Spring 4-2020

Use of a Novel Whole-Body Imaging Approach to Predict Resting Metabolic Rates in Athletes

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USE OF A NOVEL WHOLE-BODY IMAGING APPROACH TO PREDICT RESTING METABOLIC RATES IN ATHLETES

by

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A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Nutrition and Health Sciences

Under the Supervision of Professors Heather Rasmussen and Karsten Koehler

Lincoln, Nebraska

April, 2020

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University of Nebraska, 2020

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Prediction of energy expenditure allows for calculation of appropriate energy requirements, which is especially important for athletes. Resting metabolic rate (RMR) is the greatest contributor to total daily energy expenditure (TDEE) and is typically measured via indirect calorimetry. Indirect calorimetry is not always available, which results in the need for predictive equations. Most predictive equations have been developed with participants resembling the general population and have not been found to be appropriate for athletes, as they may incorrectly predict RMR due to the unique differences of body composition between athletes and the general population. The purpose of the present study was to test whether advanced segmental body composition, as measured by dual energy x-ray absorptiometry (DXA), can be utilized to more accurately predict RMR in athletes compared to previously established predictive equations. Male participants were recruited from three different sites and categorized based on body composition and energy status: sedentary controls (SED; n=33), non-weight-sensitive athletes (NWS; n=13), and weight-sensitive athletes (WS; n=55). RMR was assessed via indirect calorimetry and segmental body composition was assessed via DXA. Expanded (DXA_E) and condensed (DXA_C) DXA equations were used, in addition to three simple predictive equations (Harris-Benedict, Mifflin-St. Jeor, and Cunningham).

In SED, mean bias was found to be the lowest in DXA_E (2 kcal/d) and Cunningham (33 kcal/d) and agreement was also best (R^2=0.58) for DXA_E and Cunningham predictive equations. In athletes, mean bias was lowest in Mifflin-St. Jeor (14 kcal/d) and agreement was highest for DXA_E (R^2=0.60) and Cunningham (R^2=0.59) predictive equations. DXA_C resulted in the greatest discrimination between NWS and WS (1.00 vs. 0.92, p=0.059). Results of this study demonstrate that DXA_E is the most accurate predictive equation for SED, and DXA_E and Cunningham equations both reliably predict RMR in athletes. There is a need for future research to validate DXA_E in athletic populations, especially those experiencing a chronic state of energy deficiency.

I dedicate this thesis to my loving husband, Robert. Thank you for making this endeavor possible. I am forever grateful for your continuous support and encouragement.

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CHAPTER 1: INTRODUCTION

Resting metabolic rate (RMR) is the largest contributor of total daily energy expenditure (TDEE) and represents the energy requirements of the body's tissue compartments while at a resting state (Koehler, 2020; Melanson, 2017; Trexler et al., 2014). In athletic individuals, exercise contributes a greater percentage to TDEE compared to the general population ("Joint Statement," 2016), which impacts body composition and overall energy expenditure from RMR (Koehler, 2020).

RMR is measured in a resting, fasted state via indirect calorimetry in which the individual's inspiration of oxygen and expiration of carbon dioxide is measured to calculate energy expenditure. This measured value integrates the amount of energy utilized by all organ-tissue components of the individual while at a resting state. This method of determining energy expenditure is widely utilized and has been validated in a variety of participant populations.

Predicting an individual's RMR is important when determining energy requirements, which is especially useful for athletic populations. Athletic individuals require greater dietary energy intakes in order to function optimally and to perform the demands of their respective sport (Koehler, 2020). In assessing energy expenditure at a resting state, energy requirements can be more accurately predicted for athletes. Additionally, predicting RMR can be useful as a diagnostic tool in determining if athletes are operating in an energy deficit, or a state of low energy availability (LEA). An observable suppression of RMR is a common metabolic adaptation that occurs for athletes experiencing an energy deficit (Koehler et al., 2016, Strock et al., 2019).

RMR is typically calculated with the use of predictive equations such as Harris-Benedict, Mifflin-St. Jeor, or Cunningham (*Table 3*). Both the Harris-Benedict and Mifflin-St. Jeor equations utilize demographic and anthropometric-derived measurements to predict RMR. These predictive equations consider weight, height, age, and sex. However, these equations do not account for body composition, and specifically fat-free mass (FFM), which is one of the most impactful variables shown to influence the validity of prediction equations on RMR (Cunningham, 1980). As FFM increases, prediction equations tend to further underestimate RMR. Additionally, some predictive equations have found to overestimate RMR in obese individuals, whose FFM contributes to total body weight to a lesser degree.

The Cunningham equation uses body composition to predict RMR, specifically FFM, which is considered to be the primary predictor of RMR due to its high metabolic activity (Bosy-Westphal et al., 2004). FFM is composed of organs and tissues with varying metabolic rates, all of which contribute to total RMR. Even though Cunningham accounts for FFM, the equation does not consider the varying level of metabolic activity within FFM compartments. While prediction equations such as Harris-Benedict, Mifflin-St. Jeor, and Cunningham provide a more simplified method to estimate energy expenditure for an individual compared to indirect calorimetry, the accuracy of certain equations varies across individuals due to different factors that influence RMR.

Dual-energy x-ray absorptiometry (DXA) is considered the gold-standard method for measuring segmental body composition in individuals ("Joint Statement," 2016). This technique provides the mass for several tissue compartments within each segment of the body, which allows for a more precise comparison of the contribution of each compartment to total body composition. DXA assessments provide the amount of fat mass (FM), lean body mass or FFM, and bone mineral content (BMC) for various segments of the body including the extremities (arms and legs), trunk, and head, which enables the quantification of size for the primary tissues and organs contributing to RMR.

Previously established prediction equations using segmental body analysis derived from DXA (Bosy-Westphal, 2004; Elia, 1992) have been utilized to predict RMR in various populations (Bosy-Westphal, 2004; Koehler et al., 2016; Kosmiski et al., 2014; Müller et al., 2015; Strock et al., 2019). These equations incorporate the DXA-derived organ-tissue mass for each body segment and specific metabolic rate coefficients for each organ and tissue component. These equations account for the specific metabolic activity of each organ-tissue compartment rather than the total FFM, which results in a more specific prediction of RMR.

DXA technology is often utilized to assess body composition and bone health in athletic populations (Koehler et al., 2016; Strock et al., 2019), however there currently are not many studies exploring the validity of this DXA-derived method in predicting RMR in athletes. Overall body composition is notably different between athletes and the general population. Due to the demands of training and competition, athletes have more FFM per kilogram of body weight compared to a non-athlete or sedentary individual, resulting in a higher RMR compared to the general population (Koehler, 2020).

While body composition is considered to be the main predictor of RMR, there are additional factors that can impact RMR, including energy state. For example, athletes participating in weight-sensitive sports are more likely to experience energy deficiency compared to athletes in non-weight-sensitive sports (Burke et al., 2018; De Souza et al., 2019; Melin et al, 2019). LEA has been shown to result in RMR reduction in various populations, such as anorexia nervosa patients (Konrad et al., 2007; Kosmiski et al., 2014; Polito et al., 2000) and amenorrheic athletes (Koehler et al., 2019, Strock et al., 2019). This has been demonstrated when compared with other RMR prediction equations (e.g. Harris-Benedict, Mifflin-St. Jeor), in which measured RMR is lower than predicted RMR. When an athlete chronically experiences LEA, energy conserving adaptations occur, including the suppression of RMR.

It has not been established if the DXA-prediction method can better predict RMR in athletes experiencing LEA compared to previously utilized predictive equations. This type of prediction method could provide additional insight into the metabolic adaptations of tissue components resulting from exercising in a chronic state of energy deficiency. If the DXA-prediction method is found to more accurately predict RMR in athletic populations, this will 1) allow for more precise evidence in determining an athlete's energy requirements for their respective training and energy expenditure requirements, and 2) act as a potential diagnostic tool for athletes experiencing chronic energy deficit or found to be in a state of LEA.

Therefore, the purpose of the present study was to test whether advanced segmental body composition, as measured by dual-energy x-ray absorptiometry (DXA), can be used along with previously established organ- and tissue-specific metabolic rate coefficients to predict resting metabolic rate (RMR) in athletes. As FFM is considered to be the main predictor of RMR, we anticipate the differences in body composition relative to FFM between sedentary individuals and athletes to impact RMR.

Aim 1: To apply previously established prediction equations utilizing organ-tissue compartment mass in order to predict RMR based on advanced segmental body composition analysis in athletes and sedentary controls.

Hypothesis 1: We hypothesize that RMR can be predicted accurately in both an athletic population and sedentary controls when utilizing advanced segmental body composition analysis conducted by DXA along with previously established prediction equations.

Hypothesis 2: We hypothesize RMR predicted using advanced segmental body composition analysis conducted by DXA will more accurately reflect RMR in athletes when compared to commonly used prediction equations that utilize demographic and anthropometric measurements (e.g. Harris-Benedict, Mifflin-St. Jeor) or non-segmental-body composition (Cunningham).

Aim 2: To test whether the model of predicting RMR from advanced segmental body composition can be used for the detection of RMR suppression due to chronic energy deficiency or a state of LEA.

Hypothesis 3: We hypothesize that measured RMR will be lower than predicted RMR among athletes participating in aesthetic or weight-sensitive sports, while measured RMR will be comparable to predicted RMR in athletes engaged in non-weight-sensitive sports.

Hypothesis 4: We hypothesize that the prediction of RMR from DXA will provide better differentiation in the measured-to-predicted RMR ratio between athletic groups compared to commonly used prediction equations that utilize demographic and anthropometric measurements.

CHAPTER 2: LITERATURE REVIEW

Energy Balance and Estimating Energy Needs in Athletes

An athlete's energy requirements are dependent on training and competition load, which will vary throughout the year based on training volume and intensity. High-performance athletes are a unique population due to their elevated energy requirements (Koehler, 2020). Energy balance occurs when total energy intake is equivalent to the total daily energy expenditure (TDEE), which consists of the summation of energy expenditure from resting metabolic rate (RMR), the thermic effect of food, and the thermic effect of exercise ("Joint Statement," 2016; Melanson, 2017; Trexler et al., 2014).

Estimating dietary energy requirements has become a common practice for health professionals and registered dietitians, especially for athletic populations. Commonly utilized techniques for measuring energy expenditure in sedentary or moderately active individuals can be applied to athletes, however, there are certain considerations that should be taken into account. The most known method of estimating energy needs is by establishing RMR, since it contributes approximately 60-80% of TDEE (Elliot et al., 1989; "Joint Statement," 2016; Konrad et al., 2007; Melanson, 2017; Mifflin et al., 1990) in the general population. However, RMR may only account for 38-47% of TDEE for high-performance athletes, particularly elite endurance athletes, due to a greater energy expenditure from exercise, which can account for upwards of 50% of TDEE ("Joint Statement," 2016). The contribution of exercise to TDEE is much greater in athletes compared to the general population.

Athletes tend to have a greater proportion of fat-free mass (FFM) when compared to their non-athlete counterparts. FFM is comprised of relatively low metabolically active skeletal muscle mass (SMM) and highly metabolically active internal organs (e.g. heart, kidneys, liver, spleen). FFM is considered to be the best predictor of RMR (Cunningham, 1980; Mifflin et al., 1990) due to its highly metabolically active tissue, particularly the visceral organs (Kirstorp et al., 2000, Müller et al., 2001). With a greater ratio of FFM per kg of total body weight, it would be expected that the RMR of an athlete is greater than that of a sedentary individual or non-athlete due to the greater contribution of energy from FFM.

In understanding overall energy expenditure, this allows for more accurate estimation of energy requirements. RMR is utilized as a method for prescribing dietary energy intakes for athletes. It is important to predict RMR accurately, which reduces the under or overprediction of TDEE (Kirstorp et al., 2000), and therefore energy requirements. Significant under or overprediction of energy requirements in athletes can be detrimental to health status and overall sport performance ("Joint Statement," 2016). The most common measurement to determine RMR is via indirect calorimetry, in which the continuous gaseous exchange of oxygen consumption and carbon dioxide respiration of an individual are measured in a postabsorptive state (Compher et al., 2006).

Common Predictive Equations Utilized to Predict Resting Metabolic Rate

RMR measured via indirect calorimetry has found to be a more accurate measurement than commonly used predictive equations (*Table 3*), however, many practitioners and health care professionals do not have access to the necessary equipment to obtain RMR measurements via indirect calorimetry. For this reason, several different predictive equations have been published in order to estimate RMR (Cunningham, 1980; Harris & Benedict, 1918; Mifflin et al., 1990; Owen et al., 1986; Owen et al., 1987). Certain demographic and anthropometric variables have found to be important contributors to the variance in RMR including age, height, body weight, and FFM (Cunningham, 1980; Ferraro & Ravussin, 1992; Harris & Benedict, 1918; Mifflin et al., 1990;), with the most predictive variable of RMR being lean body mass (LBM), which accounts for up to 70% of the variability of energy expenditure from RMR (Cunningham, 1980; Sparti et al., 1997). Many predictive equations attempt to decrease the variability of RMR prediction by accounting for these variables within the equation.

The Harris-Benedict and Mifflin-St. Jeor predictive equations both utilize demographic and anthropometric-derived measurements including age, sex, height, and weight. Even though both the Harris-Benedict and Mifflin-St. Jeor equations utilize similar factors in estimating RMR, differences exist in how the equations were derived, specific to the participant population. The Harris-Benedict equations were derived from male and female subjects who were all considered to have good overall health and were representative of the general population (Harris & Benedict, 1918). Mifflin-St. Jeor equations were derived from male and female participants who were classified as normal weight or obese (defined as >120% ideal body weight). The mean weights of participants in the initial study by Harris & Benedict (1918) were much lower (males 64 kg, females 56.5 kg) compared to the mean weight of participants in the study by Mifflin et al. (1990) (males 87.5 kg, females 70.2 kg). Additionally, body composition was different between participant groups. Harris & Benedict (1918) included normal, healthy adults, whereas

Mifflin et al. (1990) included overweight and obese participants (defined as mean body mass index [BMI] values of 26 kg/m^2 for females and 27 kg/m^2 for males). Mean ages were significantly lower for participants in the Harris & Benedict (1918) study (21 to 70 years) compared to participants in the Mifflin et al. (1990) study (19-78 years). Additionally, it should be noted that Harris & Benedict (1918) derived their predictive equations from measurements of basal heat production, or basal metabolic rate (BMR), which is considered to be approximately 10% lower than RMR ("Joint Statement," 2016). The variables taken into account for both equations focus specifically on anthropometrics. Both equations account for total body weight, but the equations do not account for the proportion of FFM. Due to athletes having a greater proportion of FFM per kg of body weight, these equations (which do not consider FFM) are not potentially suitable for this population.

The Harris-Benedict equations have been reported to overpredict measured RMR by an average of 15% or greater in the general population (Mifflin et al., 1990). Daly et al. (1985) indicated that the Harris-Benedict equation overestimated energy expenditure by approximately 10-15% in their participant population of healthy men and women. It was additionally found that the Harris-Benedict equation overpredicted measured RMR in healthy women by 7-24% (Owen et al., 1986) and by 9.2% in healthy males (Owen et al., 1987). Mifflin et al. (1990) demonstrated that both the Harris-Benedict and Cunningham equations significantly overestimated RMR in healthy normal-weight and obese participants by 5% and 14-15%, respectively. However, the participant population had a greater mean weight and age compared to participants in the Harris-Benedict (1918) study, which could explain the overprediction of measured RMR for this study

(Mifflin et al., 1990). All of these studies demonstrated that body weight and FFM highly correlated with RMR (Mifflin et al., 1990).

The 1980 Cunningham predictive equation considers LBM in predicting RMR. This equation was derived from the data of healthy adult subjects, previously published in the Harris & Benedict (1918) study. Age, height, and body weight values were taken from the previously published data in order to estimate LBM with equations derived from Moore et al. (Cunningham, 1980). It was determined that sex and age are both influencing factors for body composition, but body composition was the main determinant of RMR, specifically LBM. The contribution in age was shown due to changes in body composition (Cunningham, 1980; Wang et al., 2010). Similarly, the contribution of sex can be explained by body composition differences between males and females, specifically in LBM since females tend to have a smaller proportion of FFM and greater FM when compared to males (Mifflin et al., 1990; Müller et al., 2001; Owen et al., 1987). Cunningham concluded that FFM was the single best determinant of RMR for a wide range of body compositions (Ferraro & Ravussin, 1992).

Although the equation derived from Cunningham (1980) considers the proportion of FFM, it fails to account for the varying level of metabolic activity within the different FFM compartments. Skeletal muscle is the largest tissue within the body, accounting for approximately 40% of adult body weight. However, the estimated metabolic activity of skeletal muscle is low, so its contribution to total energy expenditure is about 20-25%. Internal organs possess a much higher metabolic activity, contributing approximately 60% of total RMR, even though these tissues only account for about 5-6% of total body weight (Elia, 1992). A greater risk of inaccurately predicting RMR exists when the predictive equation does not consider these highly metabolic tissues and organs. This is especially true for athletes who have a greater proportion of FFM per kg of total body weight.

The majority of these predictive equations were initially developed using a sedentary or moderately active participant population, and for the equations developed with active individuals, the level of activity was not clearly specified. Thompson & Manore (1996) found that many predictive equations underpredicted RMR in highly trained male and female athletes, with the exception of the Cunningham equation. When assessing individual RMR values, the Cunningham was the only equation that predicted measured RMR within 158 kcal/d for male athletes and 103 kcal/d for female athletes. Even though Mifflin et al. (1990) reported an equation utilizing FFM, this equation underpredicted measured RMR of athletes by an average of 207 kcal/d for males and 184 kcal/d for females (Thompson & Manore, 1996). This evidence further demonstrates the need for a better predictive equation for RMR in athletes.

Variance in Interindividual Resting Metabolic Rate

As shown above, when utilizing a prediction equation for a different participant population than that of which the equation was originally derived, this will ultimately lead to greater variance in predicting measured RMR (Thompson & Manore, 1996). Findings from Thompson & Manore (1996) suggest that an accurate prediction of RMR in athletes cannot be determined using equations developed on less active participants. The Harris-Benedict equation was developed with participants in presumably good health that were assumed to be representative of the general population (Harris & Benedict, 1918), which explains the underestimation of measured RMR when used for an athletic population. Mifflin-St. Jeor equations most accurately predict energy expenditure in normal-weight and moderately overweight men and women (Mifflin et al., 1990), which is not an appropriate comparison for an athletic population. Body composition of athletes is different when compared to sedentary individuals or non-athletes. Athletes possess a greater ratio of FFM to FM and generally have a greater contribution of SMM, both of which impact RMR values. For this reason, athletes generally yield a greater measured RMR compared to sedentary individuals.

Another variable accounting for a portion of the variance in RMR for both men and women is energy intake and expenditure. It was found that RMR was elevated in individuals that expended large amounts of energy but matched this expenditure with energy intake. In contrast, RMR was found to be lower in athletes that consumed less energy and expended less energy (Thompson & Manore, 1996). Similar findings showed that low energy intakes can suppress RMR, which suggests that energy intake impacts RMR in active individuals who have varying levels of energy intake and expenditure (Koehler, 2020; Thompson & Manore, 1996). In another study (Bosy-Westphal et al., 2004), it was found that measured and predicted RMR values were significantly related to macronutrient intake. Dietary fat intake resulted in a positive correlation to RMR, while carbohydrate intake showed an inverse association. It was estimated that 11-12% of the difference in measured RMR compared to predicted RMR was explained by fat or carbohydrate intake (Bosy-Westphal et al., 2004).

Other studies (Konrad et al., 2007; Kosmiski et al., 2014; Müller et al., 2015; Polito et al. 2000) have investigated the impact of RMR in underweight populations and individuals experiencing caloric restriction. Measured RMR is commonly found to be lower than predicted RMR in underweight (BMI <18.5 kg/m^2) individuals, even after adjusting for FFM (Bosy-Westphal et al., 2004; Konrad et al., 2007; Kosmiski et al., 2014; Müller et al., 2015). Polito et al. (2000) found that patients with anorexia nervosa (AN) had significantly lower RMR compared to control participants. Higher RMR values were observed in rehabilitated AN patients compared to the patients currently diagnosed with AN, but RMR was still 7% lower than that of control participants. Results suggested that 62% of the variance in RMR was due to differences in body weight (Polito et al., 2000). Kosmiski et al. (2014) found similar results when comparing measured and predicted RMR between patients diagnosed with AN and healthy lean control participants. Both measured and predicted RMR were significantly lower in AN patients compared to healthy lean controls. Measured RMR was 536 kcal/d lower on average in patients with AN compared to healthy lean controls, even though it was estimated that RMR should differ between participant groups by 261 kcal/d due to differences in body composition. These results are suggestive of an adaptive suppression of metabolism in FFM. It was concluded that chronic starvation secondary to AN is accompanied by a significant reduction in the metabolic rate of FFM (Kosmiski et al., 2014).

Similarly, a study assessing caloric restriction and refeeding (Müller et al., 2015) found that starvation-induced loss of FFM adds to the variance in adaptive responses in RMR. Within a three-week period of supervised caloric restriction (consisting of a reduction in 50% of energy requirements), substantial reduction in SMM, liver, and kidney masses were observed in healthy lean male subjects. During the caloric restriction period, an average weight reduction of 7.5% occurred, with a 17.8% decrease in FM.

Loss of FFM was explained by significant losses of SMM and organ masses, which were significant for the liver and kidneys. A total of 72% of the loss in organ mass was explained by the reduced mass of the liver and kidneys. RMR decreased during the caloric restriction period and was underpredicted by 72 ± 115 kcal/d. An average FM gain of 10% was observed in participants during a two-week period of refeeding (consisting of an additional 50% of energy requirements). Participants regained 4.5% of body weight and organ-tissue masses regained to baseline levels. Measured RMR was found to increase during refeeding period above that of the baseline measurement (Müller et al., 2015).

Utilization of Dual Energy X-Ray Absorptiometry in Predicting Resting Metabolic Rate

As previously stated, body composition differences impact measured RMR, primarily in relation to FFM proportion and distribution. Even though FFM is considered to be the best determinant of RMR, the composition of the FFM contributes to the variance in RMR (Bosy-Westphal et al., 2004; Kirstorp et al., 2000, Müller et al. 2001). FFM consists of SMM with a low specific metabolic rate, as well as visceral organs with exponentially higher metabolic rates (Elia, 1992). DXA-derived measurements provide a segmental body analysis of each organ-tissue compartment. LBM in the trunk region has been shown to be a superior predictor of RMR when compared to LBM within the extremities (Kirstorp et al., 2000). This is likely due to the location of highly metabolic organs in the trunk compared to the less metabolically active SMM in the extremities.

Elia (1992) identified specific resting metabolic rate coefficients (in kcal/kg/d) for the major organs and tissue components in adults with normal weight including 240 for brain, 13 for SMM, 2.3 for bone, 4.5 for adipose tissue, and 43 for residual (*Table 2*). Residual mass is calculated as the difference of total body weight from all other organ-tissue masses. DXA-derived masses for each of the five organ-tissue compartments are multiplied by the established metabolic coefficient for each respective compartment (see *Table 1* for equations). The daily energy expenditure (in kcal/d) is then calculated as the summation of energy expenditure from all organ and tissue components (Elia, 1992). Bosy-Westphal et al. (2004) utilized MRI technology along with DXA-derived LBM of the trunk in order to develop predictive equations for several highly metabolic internal organs including the heart, kidneys, liver, and spleen. These MRI-derived equations allow for the energy estimation of these highly metabolically active organs separate from the residual mass. A separate metabolic coefficient was established for each of the MRI-derived organ masses (*Table 2*). These published equations will be utilized for the present study along with DXA technology to predict measured RMR (Bosy-Westphal et al., 2004; Elia, 1992).

Several studies (Bosy-Westphal et al., 2004; Müller et al., 2001; Wang et al., 2012) have assessed the ability to predict RMR in various populations with the utilization of DXA-derived organ-tissue mass and metabolic activity coefficients, along with previously published equations (Bosy-Westphal et al., 2004; Elia, 1992). Majority of these studies include young, healthy male and female participants, and several studies included obese individuals as controls (Bosy-Westphal et al., 2004; Wang et al., 2012).

Müller et al. (2001) assessed the influence of LBM in the prediction of RMR using DXA technology in a small participant sample of healthy, weight stable males and females. The study aimed to test the association between RMR and DXA-derived LBM of the whole-body, trunk, and peripheral body segments. It was found that LBM accounts for 74% and 83% of trunk weight for females and males, respectively. FM was found to contribute more to peripheral weight compared to trunk weight. There was a strong relationship between whole-body LBM and RMR. Although, when RMR was expressed per kilogram of whole-body LBM, RMR was found to decrease as whole-body LBM increased. These findings suggest that with greater overall LBM, there is a decreased proportion of highly metabolically active LBM. Adjusting RMR for the ratio of trunk LBM to peripheral LBM was suggested to be beneficial for the comparison of RMR between individuals differing in whole-body LBM (Müller et al., 2001).

When assessing RMR in obese participants with the use of DXA-derived predictive equations, Wang et al. (2012) found that measured RMR was significantly lower in nonobese women compared to obese women. There was a significant correlation between measured and predicted RMR in nonobese women, however RMR was found to be overpredicted by 1.9% in obese women (Wang et al., 2012). Another study (Bosy-Westphal et al., 2004) that assessed underweight (BMI < 18.5), intermediate (BMI 19 to 28), and obese (BMI >30) participants found that RMR derived from DXA and MRI measurements of organ-tissue compartments was similar to measured RMR in all participant groups. Overprediction of RMR was observed in four obese participants and one normal weight participant, however there were not any specific trends in body composition identified for this overprediction. There were observed differences in body composition specific to organ compartments between participant groups. The masses of the liver, spleen, and kidneys were all lower in underweight participants when comparing all three participant groups. Brain and heart masses were also found to be lower in underweight participants, all of which contributed to lower measured RMR values in the underweight group. Findings of this study suggest that a decrease in organ metabolic rate does not exist when an increase in organ mass occurs (Bosy-Westphal et al., 2004), contrary to findings from Müller et al. (2001).

Detection of Energy Deficiency in Athletes

It has been previously demonstrated that athletes participating in aesthetic or weight sensitive sports are more likely to be in a state of low energy availability (LEA) (Burke et al., 2018; De Souza et al., 2019, Heikura et al., 2018; Logue et al., 2018; Melin et al., 2015; Sundgot-Borgen et al., 2010), meaning the athlete is not consuming adequate dietary energy intake in order to sustain the energy expenditure required for optimal metabolic function and required training demands ("Joint Statement," 2016; Koehler, 2020; Logue et al., 2018; Tenforde et al., 2015). When dietary energy intake is insufficient to meet metabolic demands, energy is prioritized to the physiological processes necessary for survival, and the processes considered unnecessary for survival (e.g. growth, reproduction) are suppressed as a result. A suppression of metabolism and energy expenditure is observed in these individuals (Koehler et al., 2016; Strock et al., 2019), resulting in a sequela of symptoms including increased risk for bone fractures, impact on reproductive and metabolic hormone function, and decreased performance in sport (De Souza et al., 2019; Heikura et al., 2018; Koehler et al., 2016; Melin et al., 2019; Mountjoy et al., 2014; Strock et al., 2019).

In efforts to conserve energy, various metabolic adaptations have been observed in athletes experiencing LEA (Burke et al., 2018; De Souza et al., 2019; Heikura et al., 2018; Koehler et al., 2016; Koehler et al., 2020; Logue et al., 2018; Mountjoy et al., 2014; Strock et al., 2019). An athlete experiencing an energy deficit may maintain a normal body weight due to adaptations that occur, such as suppression of RMR. The athlete may still be considered weight stable, while simultaneously experiencing impaired physiological function secondary to LEA (Logue et al., 2018; Melin et al., 2019). Reproductive hormone status has been used in previous studies (Koehler et al., 2016; Koehler, 2020; Melin et al., 2019; Strock et al., 2019; Trexler et al., 2014) with female athletes as an indicator of energy deficiency.

Strock et al. (2019) evaluated predicted RMR values from Harris-Benedict and Cunningham (both 1980 and 1991 versions) equations, along with DXA-derived prediction methods and RMR measured via indirect calorimetry to determine metabolic impacts of energy deficiency in a group of athletic females. Participants were categorized based on menstrual status. Amenorrheic athletes were found to have lower measured RMR values compared to other participant groups. Additionally, the Harris-Benedict as well as both Cunningham equations overpredicted measured RMR for these female athletes, with the Harris-Benedict equation predicting approximately 20% greater than measured RMR (Strock et al., 2019). Similarly, Koehler et al. (2016) found that female athletes exhibiting exercise-associated amenorrhea displayed lower measured RMR than women with eumenorrheic menstrual cycles when expressed relative to LBM. On average, measured RMR was determined to be 8% lower than DXA-predicted RMR in exercising women with amenorrhea. Body composition between female athletes with amenorrhea compared to eumenorrheic females revealed significant differences in adipose tissue and residual mass. There were not significant differences between masses of the brain, SMM, or bone between participant groups. Organ and tissue compartments contributing greater metabolic activity were not found to be reduced in exercising women with amenorrhea, suggesting that women with exercise-associated amenorrhea may experience metabolic suppression as an adaptive response for energy conservation (Koehler et al., 2016). These particular studies further validate RMR suppression due to metabolic adaptations presenting in menstrual irregularities and reduced reproductive hormone production.

In female athletes, there have been observed reductions in both reproductive and metabolic hormones during energy deficient states. Commonly assessed reproductive hormones in female athletes are estradiol and progesterone (Koehler et al., 2016; Strock et al., 2019). Other metabolic hormones including total triiodothyronine, insulin-like growth factor-1 (IGF-1), leptin and insulin have all shown to decrease in athletes who are expending high levels of energy (De Souza et al., 2019; Koehler et al., 2016; Trexler et al., 2014). A reduction of total triiodothyronine has been shown to strongly correlate with menstrual disturbances in female athletes (Koehler et al., 2016). Subsequently, the upregulation of cortisol, growth hormone (De Souza et al., 2019), and ghrelin has also been shown for athletes experiencing LEA (Trexler et al., 2014).

Energy deficiency is more difficult to detect in male athletes, since male reproductive function is less vulnerable to energy status (Koehler, 2020). Studies have identified changes in reproductive and metabolic hormones that are involved in the hypothalamic-pituitary-gonadal axis (Mountjoy et al., 2014; Tenforde et al., 2015). Male athletes that regularly engage in endurance training have shown to exhibit persistently reduced testosterone levels, (Heikura et al., 2018; Logue et al., 2018; Tenforde et al.,

2015) which may have potential future health implications to bone health, metabolic function, and fertility (Melin et al., 2019). Similar to observations made in female athletes (Koehler et al., 2016; Mountjoy et al., 2014), there have been observed lower pulse frequencies of luteinizing hormone and follicle-stimulating hormones in elite male athletes (Tenforde et al., 2015). There is a need for further research related to the impacts on endocrine and metabolic function due to LEA in male athletes.

Associations of energy-conserving metabolic adaptions have been observed in athletes (e.g. menstrual disturbances in addition to reproductive and metabolic hormonal imbalance or reduction), however an objective measurement of energy availability for athletes does not currently exist (Heikura et al., 2018; Logue et al., 2018; Melin et al., 2019; Strock et al., 2019). Different methods have been used to assess energy intake and energy expenditure as part of the assessment of energy availability in athletes, but these methods are not always reliable due to self-reported data (Logue et al., 2018). Previous literature has established threshold values (in kcal/kg of FFM/d) which quantify the energy availability of an athlete. A cutoff threshold value of 30 kcal/kg of FFM/d has been previously utilized for the assessment of energy availability in female athletes experiencing exercise-associated amenorrhea or hormone reductions (De Souza et al., 2019; Heikura et al., 2018; Melin et al., 2019).

Additionally, the measured-to-predicted RMR ratio has been more recently utilized as a potential indicator of energy status in several studies (Koehler et al., 2016; Strock et al., 2019). It was found that exercising women with amenorrhea demonstrated lower concentrations of total triiodothyronine and leptin, which significantly correlated with the measured-to-predicted RMR ratio (Koehler et al., 2016). A ratio threshold of

0.90 or less has been identified as an indicator of LEA (Strock et al., 2019). It is difficult to assess energy availability in athletes due to the variety of contributing factors and potential metabolic adaptations. RMR provides the greatest potential for energy conservation since it has found to be the greatest component of TDEE. In quantifying the suppression of RMR, this could serve as a potential diagnostic tool for energy deficiency in athletes (Koehler, 2020). As body composition has consistently been found to be a main contributor of RMR, an athlete's energy expenditure can be predicted from DXA-derived measurements and published equations (Bosy-Westphal et al., 2004; Elia, 1992) in order to more accurately determine energy requirements, which can potentially reduce the incidence of LEA in athletes.

There is currently limited literature in which this DXA-derived prediction method is utilized for athletic populations, specifically for athletes participating in aesthetic or weight-sensitive sports. It has been shown that a chronic state of energy deficiency results in RMR suppression, in addition to other metabolic adaptations impacting both overall health and performance in sport (Burke et al., 2018; Koehler et al., 2016; Logue et al., 2018; Mountjoy et al., 2014). If found to be an appropriate prediction method for estimating energy requirements in athletes, the DXA-predictive method could potentially be used as an indication of energy deficiency or diagnosis tool for LEA in athletic populations.

CHAPTER 3: APPROACH

Study Design

This cross-sectional study was designed to predict RMR from DXA-derived segmental body composition measurements in athletes. Three separate participant groups were included in order to address the study's aims: athletes and sedentary controls for Aim 1; and athletes categorized as participating in weight-sensitive sports and athletes in non-weight-sensitive sports for Aim 2. Data for the following study were collected at three sites: the University of Copenhagen (Denmark), the University of Agder (Norway), and the University of Nebraska-Lincoln (United States). Study protocols were approved by the local ethics committees and institutional review boards for each site. All participants provided informed consent, and details of each assessment were described to participants prior to participation in the study. For purposes of this present study, data were combined and reanalyzed for segmental body composition in order to predict RMR. All study participants performed RMR and DXA assessments, which are detailed below.

The collected data across sites were reanalyzed for each participant. RMR was predicted with multiple equations that utilize DXA-derived segmental body composition measurements along with organ- and tissue-specific metabolic activity coefficients (Bosy-Westphal et al., 2004; Elia, 1992). These predictive equations utilizing DXA-derived measurements were compared to several widely used equations that utilize general anthropometric measurements. RMR was predicted for each participant utilizing each outlined equation, and results were compared to the participants' RMR measured via indirect calorimetry.

Participants

All study participants (n=101) were Caucasian males between the ages of 20 and 60 years. Exclusion criteria from participation in the study included taking any medications that would interfere with measurements. Participants were categorized into three separate groups based on differences in body composition and energy status: sedentary controls (SED), non-weight-sensitive athletes (NWS), and weight-sensitive athletes (WS). SED (n=33) were selected from a larger data set, which was previously collected at the Department of Nutrition, Exercise and Sports at the University of Copenhagen between 2008 and 2015. NWS athletes (n=13) were recruited from varsity or club level teams at the University of Nebraska-Lincoln. WS athletes include professional ballet dancers and competitive endurance athletes. Ballet dancers (n=17) were recruited at the Royal Ballet, Copenhagen, Denmark. Endurance athletes (distances runners n =20, cyclists n=18) were recruited in Southern Norway. SED were selected based on FFM-match to athletes enrolled in the study.

Resting Metabolic Rate Measurements

RMR was measured for all participants in the morning hours between 05:00 and 09:00 using a ventilated hood system (sedentary controls and weight-sensitive athletes: Oxycon Pro 4, Germany; non-weight-sensitive athletes: Parvo Medics, USA). All systems were calibrated according to manufacturer instructions and operated by trained personnel. RMR testing occurred following an overnight fast, and participants were instructed to abstain from alcohol, caffeine, tobacco, and exercise for at least 12 hours prior to their scheduled testing. Participants were instructed to travel to the testing site via motorized transportation on the morning of their assessment. Biking or walking to the lab was not permitted in order to limit physical activity of the participant prior to the assessment. After participants were familiarized with the procedure and equipment, participants rested in a supine position for 30 minutes. The ventilated hood was placed over their head and respiratory gas exchange was measured for at least 30 minutes. RMR was calculated from steady state data.

Dual-Energy X-ray Absorptiometry

Body composition was assessed via whole-body DXA scans. All scans were performed on DXA equipment from the same manufacturer (GE Lunar Corporation) by certified personnel in accordance with local regulations. All scans were conducted in the fasted and rested state with the participant in the standardized positioning. For the scope of this study, all scans were reanalyzed using the same software (Lunar iDXA, enCore version 14.10, USA) by the same individual. The software predetermined guidelines for body composition segments. All segments were manually adjusted according to specific guidelines. See *Figure 1* for whole body DXA scan of male participant. Skull area was defined as the area superior to the proximal end of the mandible. Lean mass in the extremities were defined as the area lateral to the glenohumeral joint (arms) and distal to the femoral neck (legs). The trunk included the area medial to each glenohumeral joint down to the proximal portion of the iliac crest of the pelvis. Bone mineral content was defined within each segmental of the body in order to subtract from FFM, which would provide LBM. Body composition segments were further divided within the trunk region to define bone mineral content for each body segment. Linear separation of the spine ran medially through the trunk from the distal end of the mandible to the proximal portion of the iliac crest of the pelvis. The pelvic region included the area from the proximal end of the iliac crest to the top of each femoral neck, including the distal end of the ischium of the pelvic bone.

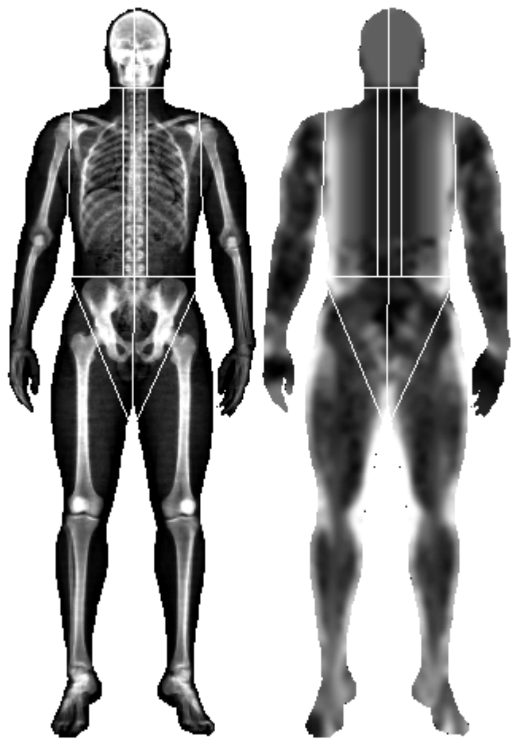

Figure 1*. Whole body dual energy x-ray absorptiometry (DXA) scan of male participant enrolled in the current study. Manually adjusted lines determining segmental body composition are displayed.*

Modeling of Organ-Tissue Mass

DXA-derived measurements were utilized to determine the organ and tissue mass for each segment of the body. Tissue components derived directly from whole body DXA measurements include FFM, bone mineral content (BMC), LBM, and FM. Skeletal muscle mass (SMM) was calculated as the sum of lean mass in the extremities (arms and legs). Skull area was defined as the head area (in cm^2) and was used to predict brain mass. Total FM and BMC measurements were used to calculate adipose mass and bone mass, respectively. While the remaining internal organ masses (kidney, heart, liver, spleen) cannot be derived directly from DXA, a previously developed equation was used to derive organ mass from LBM in the trunk through magnetic resonance imaging (MRI) technology (Bosy-Westphal et al., 2004), which are outlined in *Table 1*. Residual mass was calculated by subtracting the sum of all organ-tissue masses from total body weight.

Two different equations that utilize DXA-derived measurements to predict RMR were compared in this study. Both DXA-derived equations consider the following organ-tissue compartments: brain, skeletal muscle, bone and adipose tissue, and residual mass. The expanded DXA-predictive equation (DXA_E) will include the highly metabolic internal organs (kidney, heart, liver, spleen) separate from the residual mass, with a respective metabolic activity coefficient for each organ. The condensed DXA-predictive equation (DXA_C) incorporates these internal organs within the calculation of residual mass. Both DXA-predictive equations are included in the study in order to assess the variation of these highly metabolically active internal organs in predicting RMR in sedentary individuals and athletes. Calculation equations for each organ-tissue compartment are found in *Table 1*.

Table 1. Calculation of Mass (kg) for Each Organ-Tissue Compartment

Organ-Tissue Compartment	Equation to Calculate Mass (kg)
Brain	= 0.005 x skull area + 0.2 x sex* + 0.24
Skeletal Muscle	= lean tissue in extremities x 1.13 – 0.02 x age + 0.61 x sex* + 0.97
Bone	= 1.85 x bone mineral content
Adipose Tissue	= 1.18 x fat mass
Residual (DXA_C)	= body weight – (brain + skeletal muscle + bone + adipose tissue)
Heart	= 0.012 (LBM of trunk)$^{1.0499}$
Kidney	= 0.0165 (LBM of trunk)$^{0.9306}$
Liver and Spleen	= 0.0749 (LBM of trunk)$^{0.9426}$
Residual (DXA_E)	= body weight – (brain + skeletal muscle + bone + adipose tissue + heart + kidney + liver/spleen)

**Where sex = 0 for female and 1 for male*
LBM, lean body mass
LBM derived via DXA measurements

Prediction of Resting Metabolic Rate from Organ-Tissue Mass

The energy expenditure of each organ-tissue compartment (in kilocalories per kilogram per day) was calculated by multiplying the mass of each compartment by a specific metabolic activity coefficient that is representative of the metabolic rate for that respective organ-tissue compartment (*Table 2*). These metabolic activity coefficients have been previously published (Bosy-Westphal et al., 2004; Elia, 1992). Predicted RMR was then calculated as the sum of all organ-specific metabolic rates (kcal/d), with the internal organs accounted for separately with the DXA_E equation and included within residual mass calculation for the DXA_C equation, as shown below:

DXA_E = (240 x brain mass) + (13 x SMM) + (2.3 x bone mass) + (4.5 x adipose tissue mass) + (441 x heart mass) + (441 x kidney mass) + (201 x liver/spleen mass) + (6.9 x residual mass)

DXA_C = (240 x brain mass) + (13 x SMM) + (2.3 x bone mass) + (4.5 x adipose tissue mass) + (43 x residual mass)

Table 2. Previously Established Metabolic Activity Coefficients for Each Respective Organ-Tissue Compartment to Calculate Organ-Specific Energy Expenditure (kcal/kg/d)

Organ-Tissue Compartment	**DXA_E Metabolic Activity Coefficients (kcal/kg/d)**	**DXA_C Metabolic Activity Coefficients (kcal/kg/d)**
Brain	240	240
Skeletal Muscle	13	13
Bone	2.3	2.3
Adipose Tissue	4.5	4.5
Heart	441	-
Kidney	441	-
Liver and Spleen	201	-
Residual	6.9	43

DXA_C Metabolic activity coefficients established by Elia 1992
DXA_E Metabolic activity coefficients established by Bosy Westphal et al. 2004

Other Predictive Equations

Several widely used equations have previously been established in order to predict RMR (Cunningham, 1980; Harris & Benedict, 1918; Mifflin et al., 1990). The predictive equations that will be utilized for purposes of this study in comparing the ability to accurately predict RMR for athletes and sedentary controls include Cunningham (1980), Mifflin-St. Jeor, and Harris-Benedict equations (*Table 3*). These predictive equations all utilize demographic and anthropometric-derived measurements. The Cunningham equation considers LBM, whereas the Mifflin-St. Jeor and Harris-Benedict equations both consider age, sex, height (cm), and weight (kg).

Table 3. Predictive Equations Commonly Utilized to Calculate RMR

Predictive Equation	
Cunningham	= 500 + (22 x LBM)
Mifflin-St. Jeor*	= 9.99(W) + 6.25(H) – 4.92(A) + 5
Harris-Benedict*	= 13.75(W) + 5.003(H) – 6.775(A) + 66.47

**Only predictive equation for male is listed*
LBM, lean body mass in kg; W, weight in kg; H, height in cm; A, age in years
LBM derived via DXA measurements

Statistical Analysis

Statistical analyses were conducted using Microsoft Excel (version 16.34, 2020). Group differences between participant groups (SED vs. athletes and NWS vs. WS) were assessed to identify significant differences in demographics and segmental body composition using two-tailed *t* tests. To address aim 1, regression analysis and Bland-Altman plots (Bland & Altman, 1986) were used to compare the relationship between predictive equations and measured RMR within SED and athletes. Linear regression equations were assessed for slope and intercept. Slope values closer to one and intercept values closer to zero were deemed preferential. Coefficients of determination (R^2) were assessed to explain variance between each predictive equation and measured RMR. R^2 values closer to a value of one were considered preferential in explaining the variance between the predictive equation and measured RMR. All prediction equations were plotted against the participants' measured RMR values in order to assess the agreement between prediction methods. Additionally, the mean bias was calculated to explain the accuracy of each prediction equation in predicting measured RMR. Mean bias was calculated as the mean of the difference between predicted and measured RMR, with positive values indicating the RMR was underestimated and negative values indicating that RMR was overestimated. Further, 95% limits of agreement were determined to assess the precision of each predictive equation. The upper and lower end of the 95% limits of agreement were calculated as mean bias ± 1.96 times the standard deviation of the difference between predicted and measured RMR. To address aim 2, the ratio of measured RMR to predicted RMR was calculated for all five predictive equations in NWS and WS athletes. Two-tailed *t* tests were utilized to determine significance of discrimination in RMR ratios between NWS and WS athletes.

CHAPTER 4: RESULTS

Participants

Participant demographics are presented in *Table 4*. All study participants were Caucasian males between the ages of 20 and 60 years. The mean age was significantly higher in SED when compared to athletes ($p<0.001$), while WS athletes were significantly older than NWS athletes ($p<0.001$). Mean height was not significantly different between SED and all athletes ($p=0.18$), or between NWS and WS athletes ($p=0.39$). SED had significantly higher body weight when compared to all athletes ($p<0.001$), and NWS athletes weighed significantly more when compared to WS athletes ($p<0.001$).

Body composition details are presented in *Table 4* for each participant group. Body mass index (BMI) was significantly higher in SED when compared to all athletes ($p<0.001$), and BMI was significantly higher in NWS when compared to WS athletes ($p<0.001$). LBM was similar between SED and all athletes ($p=0.20$), while lean mass index (LMI), calculated as the ratio of total lean mass derived by height squared, was significantly higher in SED when compared to all athletes ($p=0.008$). LBM and LMI were both significantly higher in NWS compared to WS athletes (both $p=0.001$). FM as well as body fat percentage were significantly higher in SED compared to all athletes (both $p<0.001$). FM tended to be higher in NWS athletes when compared to WS ($p=0.094$), while body fat percentage was not significantly different between athlete groups ($p=0.50$).

Table 4. Participant Demographics and Body Composition of All Participants

	Sedentary		All Athletes		Non-Weight-Sensitive		Weight-Sensitive	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Age, year	41.8 ± 10.0**	21.0–60.5	31.2 ± 9.3	20.1–51.8	23.1 ± 2.3	20.3–29.6	33.1 ± 9.26† †	20.1–51.8
Weight, kg	105 ± 13.4**	80.5–132	76.4 ± 8.7	58.0–102	88.1 ± 9.7† †	73.1–102	73.7 ± 5.59	58.0–86.0
Height, cm	180 ± 6.7	161–193	182.1 ± 6.4	169–201	184 ± 8.1	169–201	182 ± 5.96	169–193
BMI, kg/m^2	32.3 ± 3.0**	27.3–38.3	23.1 ± 2.4	18.5–29.4	26.1 ± 2.0† †	23.5–29.4	22.4 ± 1.80	18.5–25.9
^Total LBM, kg	63.6 ± 6.2	51.3–74.1	61.9 ± 6.8	44.0–84.1	69.8 ± 8.4†	58.7–84.1	60.0 ± 4.78	44.0–73.2
^LMI, kg/m^2	19.6 ± 1.5*	16.4–22.7	18.7 ± 1.8	15.4–24.9	20.7 ± 2.0†	17.1–24.9	18.2 ± 1.37	15.5–22.6
^Total FM, kg	37.9 ± 8.8**	24.2–56.7	12.0 ± 4.0	7.13–30.0	14.6 ± 6.1†	9.0–30.0	11.4 ± 3.18	7.13–19.9
^Body Fat, %	35.8 ± 4.6**	26.6–46.6	15.5 ± 4.3	9.8–29.5	16.4 ± 5.9	10.6–29.5	15.3 ± 3.82	9.8–23.9

^Body composition derived via DXA measurements
BMI, body mass index; LBM lean body mass; LMI, lean mass index; FM, fat mass
**P<0.1, **P<0.001 for sedentary and all athletes*
† P<0.1, † † P<0.001 for non-weight-sensitive athletes and weight-sensitive athletes

Measured RMR

Measured RMR was 1,867 ± 232 kcal/d for SED, which was significantly higher than measured RMR in all athletes (1,739 ± 231 kcal/d, $p=0.012$). Measured RMR was also significantly higher in NWS athletes (2,009 ± 341 kcal/d) when compared to WS athletes (1,676 ± 136 kcal/d, $p=0.004$).

RMR Prediction from DXA Analysis

When using the expanded DXA-predictive equation (DXA_E), predicted RMR for SED was 1,869 ± 147 kcal/d, which was significantly higher compared to all athletes (1,776 ± 147 kcal/d, $p=0.004$). NWS had significantly higher DXA_E predicted RMR (1,937 ± 177 kcal/d) compared to WS athletes (1,738 ± 110 kcal/d, $p=0.002$). When using the condensed DXA-predictive equation (DXA_C), predicted RMR was significantly lower for SED (1,775 ± 127 kcal/d) compared to all athletes (1,863 ± 169 kcal/d, $p=0.004$). DXA_C predicted RMR was also found to be higher in NWS (2,012 ± 219 kcal/d) compared to WS athletes (1,828 ± 136 kcal/d, $p=0.012$).

Segmental body composition data is presented in *Table 5* for all participant groups. When comparing segmental body composition analyzed via DXA technology between SED and all athletes, SED had significantly more total adipose tissue ($p<0.001$) and SMM ($p=0.008$). Bone mass was similar between SED and athletes ($p=0.235$). Athletes had significantly greater brain mass, as well as residual mass, both for the expanded and condensed approach, compared to SED (all $p<0.001$). Masses of internal organs were similar between SED and athletes (all organs $p\geq0.131$).

When comparing segmental body composition analyzed via DXA technology between NWS and WS athletes, NWS athletes had significantly greater SMM ($p<0.001$). There were also trends indicating that adipose tissue mass ($p=0.094$) and bone mass ($p=0.059$) were greater in NWS compared to WS. Brain mass tended to be greater in WS compared to NWS athletes ($p=0.065$). The internal organ masses including the heart, kidney, liver, and spleen were all significantly greater in NWS when compared to WS athletes (all $p=0.008$). Residual mass was similar in NWS athletes when compared to WS for both DXA_E residual mass ($p=0.174$) and DXA_C residual mass ($p=0.137$).

Energy expenditure for each organ-tissue compartment is presented in *Table 6*. Organ-tissue compartments that were observed to be significantly greater in mass for a participant group were also found to contribute significantly greater energy expenditure for that respective participant group.

Table 5. Segmental Body Composition Analysis by DXA for All Participant Groups

Organ-Tissue Compartment Mass (kg)	**Sedentary (n=33)**	**Non-Weight-Sensitive (n=13)**	**Weight-Sensitive (n=55)**
Brain	1.60 ± 0.08**	1.67 ± 0.05	1.70 ± 0.07†
Skeletal Muscle	35.9 ± 4.67*	39.1 ± 5.01††	31.8 ± 2.71
Bone	6.21 ± 0.65	6.40 ± 0.72†	5.97 ± 0.47
Adipose Tissue	44.7 ± 10.4**	17.2 ± 7.15†	13.5 ± 3.75
Residual (DXA_C)	16.5 ± 2.03**	23.5 ± 3.99	21.7 ± 2.54
Heart	0.41 ± 0.04	0.47 ± 0.06†	0.41 ± 0.04
Kidney	0.38 ± 0.03	0.42 ± 0.05†	0.38 ± 0.03
Liver and Spleen	1.79 ± 0.14	2.00 ± 0.23†	1.80 ± 0.15
Residual (DXA_E)	14.0 ± 1.92**	20.6 ± 3.67	19.1 ± 2.32

All values are presented as mean ± SD
**P<0.1, **P<0.001 for sedentary and all athletes*
†P<0.1, ††P<0.001 for non-weight-sensitive athletes and weight-sensitive athletes

Table 6. Energy Expenditure by Organ-Tissue Compartment for All Participant Groups

Energy Expenditure by Compartment (kcal/d)	**Sedentary (n=33)**	**Non-Weight-Sensitive (n=13)**	**Weight-Sensitive (n=55)**
Brain	385 ± 19.3**	401 ± 11.2	408 ± 17.5†
Skeletal Muscle	466 ± 60.7*	508 ± 65.2††	414 ± 35.3
Bone	14 ± 1.49	14.7 ± 1.65†	13.7 ± 1.07
Adipose Tissue	201 ± 46.6**	77 ± 32.2†	61 ± 16.9
Residual (DXA_C)	709 ± 87.2**	1011 ± 172	932 ± 109
Heart	181 ± 15.4	206 ± 26.2†	182 ± 17.1
Kidney	167 ± 12.5	186 ± 21.0†	168 ± 14.0
Liver and Spleen	359 ± 27.4	402 ± 46.0†	361 ± 30.5
Residual (DXA_E)	96 ± 13.2**	142 ± 25.3	132 ± 16.0

All values are presented as mean ± SD
**P<0.1, **P<0.001 for sedentary and all athletes*
†P<0.1, ††P<0.001 for non-weight-sensitive athletes and weight-sensitive athletes

Comparison of DXA-Prediction Equations in Predicting RMR

DXA_E predicted measured RMR in SED with a mean bias of 2 kcal/d, with 95% limits of agreement ranging from -301 to 297 kcal/d (*Figure 2* and *Table 7*). In athletes, the mean bias for DXA_E was 37 kcal/d, with 95% limits of agreement ranging from -331 to 256 kcal/d. Linear regression between DXA_E predicted RMR and measured RMR (*Figure 3*) revealed coefficients of determination of R^2=0.58 and R^2=0.60 in SED and athletes, respectively. The slope of the linear regression was m=0.48 (SED) and m=0.49 (athletes), and the intercept was 965 kcal/d (SED) and 921 kcal/d (athletes).

DXA_C predicted measured RMR in SED with a mean bias of 92 kcal/d, with 95% limits of agreement ranging from -234 to 417 kcal/d (*Figure 4*). The mean bias for DXA_C in athletes was 124 kcal/d, with 95% limits of agreement ranging from -431 to 183 kcal/d. Linear regression between DXA_C predicted RMR and measured RMR (*Figure 5*) revealed coefficients of determination of R^2=0.51 (SED) and R^2=0.54 (athletes). The slope of the linear regression was m=0.39 and m=0.54 for SED and athletes, respectively. The intercept was 1,043 kcal/d n SED and 926 kcal/d in athletes.

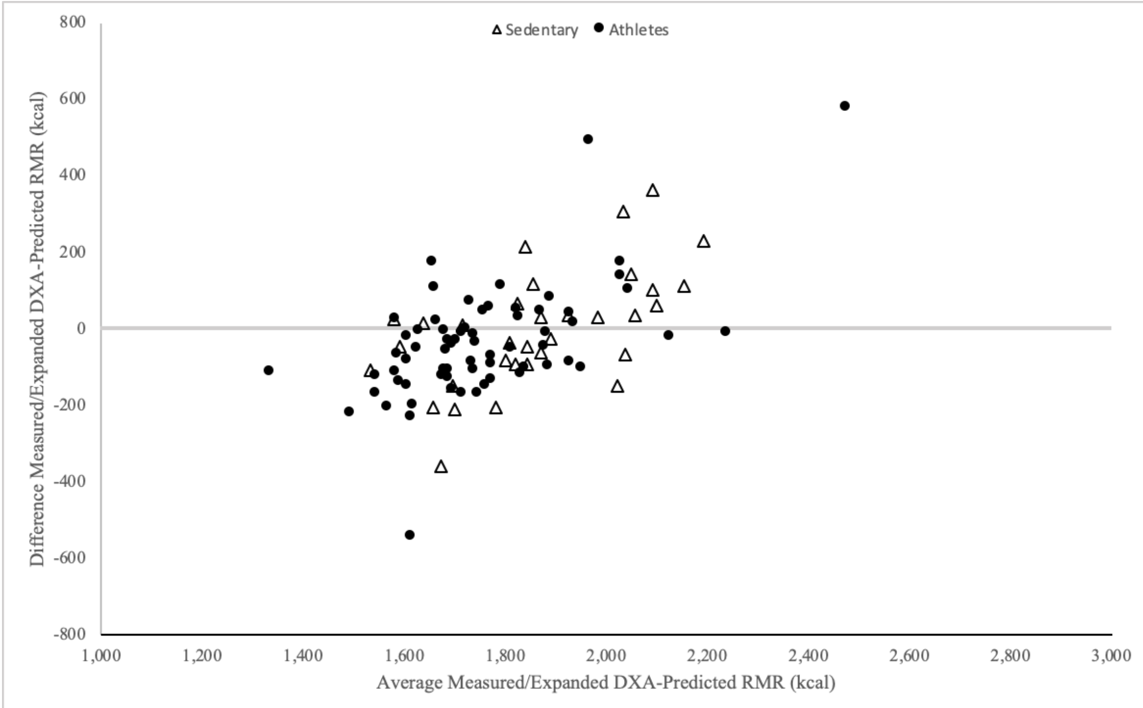

Figure 2. *Bland-Altman plot of measured RMR vs. RMR predicted by the expanded DXA-predictive equation in sedentary controls (open triangles) and athletes (closed circles). The grey line indicates a mean bias of 0 kcal/d.*

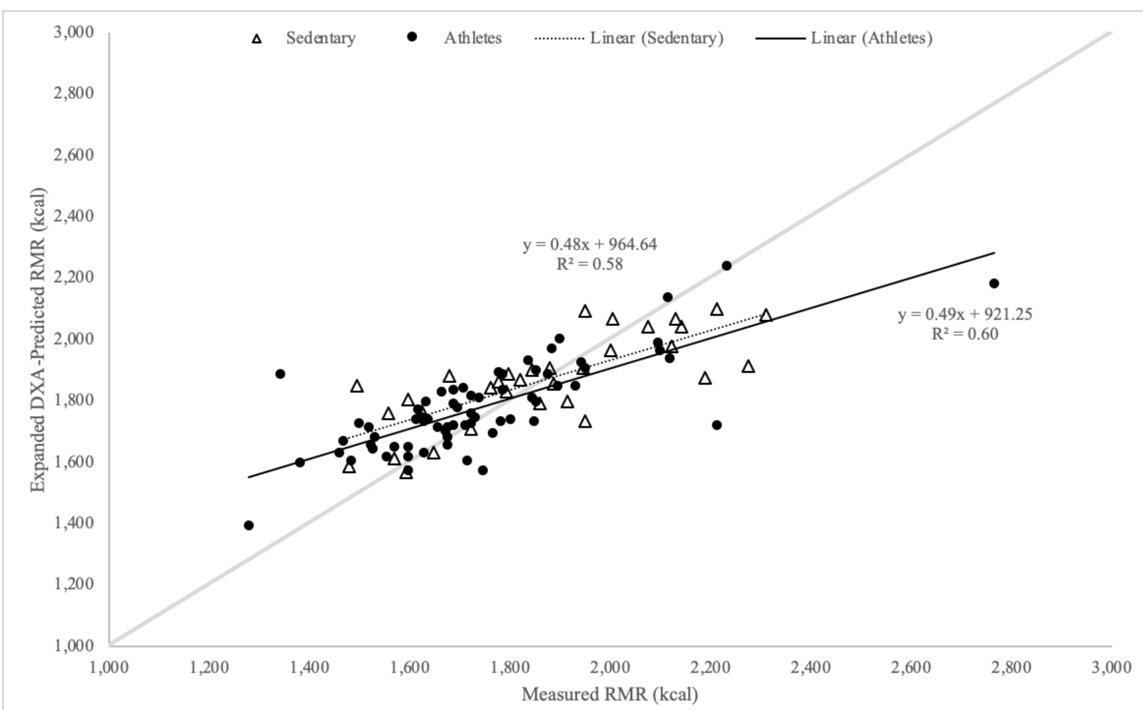

Figure 3. *Comparison of measured RMR and RMR predicted using the expanded DXA-predictive equation in sedentary controls (open triangles) and athletes (closed circles). The grey line denotes the line of identity.*

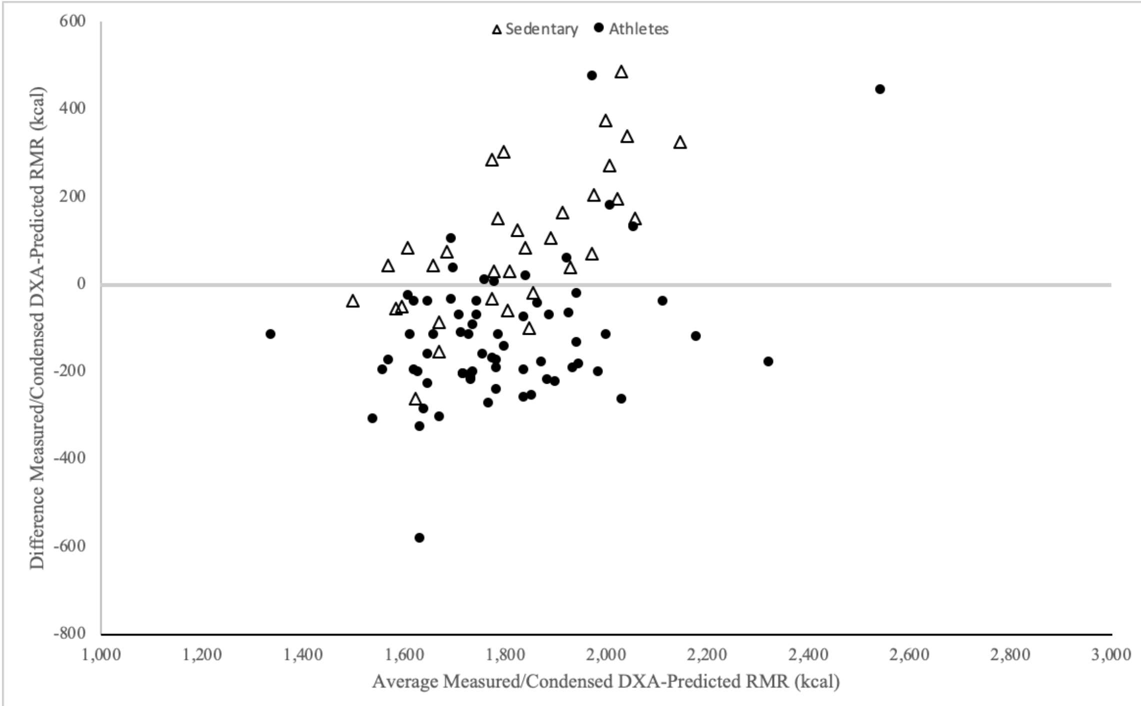

Figure 4. *Bland-Altman plot of measured RMR vs. RMR predicted by the condensed DXA-predictive equation in sedentary controls (open triangles) and athletes (closed circles). The grey line indicates a mean bias of 0 kcal/d.*

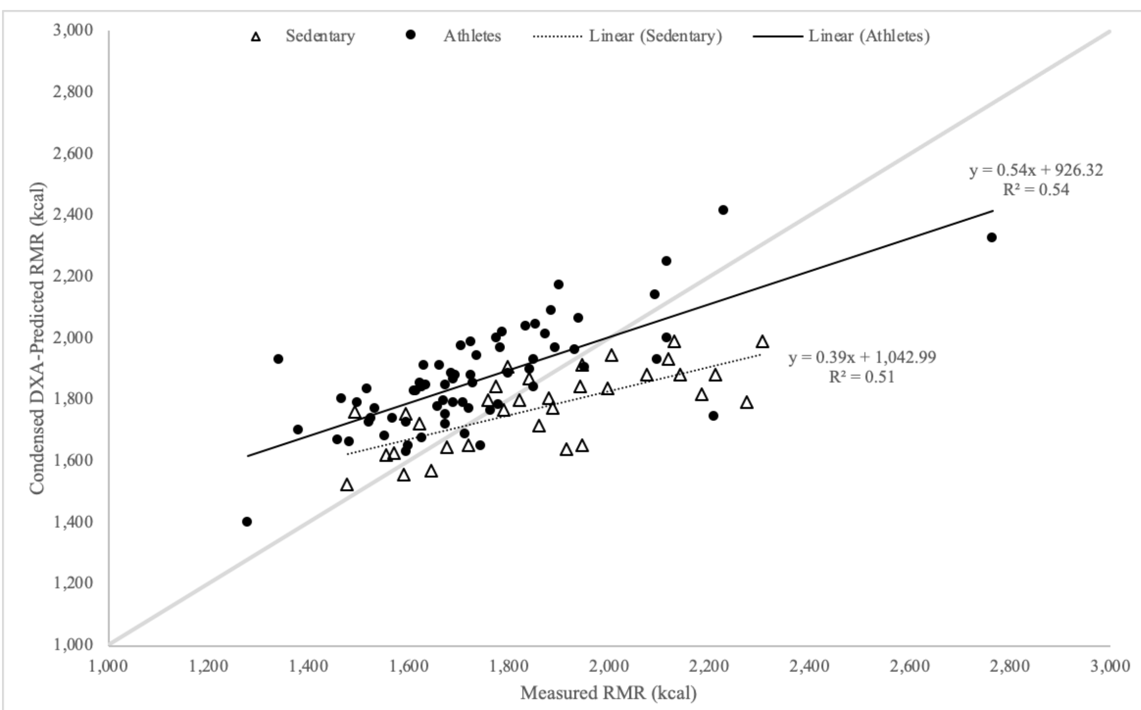

Figure 5. *Comparison of measured RMR and RMR predicted using the condensed DXA-predictive equation in sedentary controls (open triangles) and athletes (closed circles). The grey line denotes the line of identity.*

Comparison of Other Predictive Equations in Predicting RMR

The Harris-Benedict predictive equation predicted measured RMR in SED with a mean bias of 262 kcal/d, with 95% limits of agreement ranging from -642 to 118 kcal/d (*Figure 6* and *Table 7*). In athletes, the mean bias for Harris-Benedict was 78 kcal/d, with 95% limits of agreement ranging from -379 to 224 kcal/d. Linear regression between the Harris-Benedict predicted RMR and measured RMR (*Figure 7*) revealed coefficients of determination of R^2=0.44 and R^2=0.56 in SED and athletes, respectively. The slope of the linear regression was m=0.69 (SED) and m=0.51 (athletes), and the intercept was 832 kcal/d in SED and 931 kcal/d in athletes.

The Mifflin-St. Jeor predictive equation predicted measured RMR in SED with a mean bias of 108 kcal/d, with 95% limits of agreement ranging from -457 to 242 kcal/d (*Figure 8*). The mean bias in athletes for Mifflin-St. Jeor was 14 kcal/d, with 95% limits of agreement ranging from -327 to 300 kcal/d. Linear regression between the Mifflin-St. Jeor predicted RMR and measured RMR (*Figure 9*) revealed coefficients of determination of R^2=0.43 in SED and R^2=0.56 in athletes. The slope of the linear regression was m=0.54 and m=0.40 for SED and athletes, respectively, and the intercept was 970 kcal/d (SED) and 1,049 kcal/d (athletes).

The Cunningham predictive equation predicted measured RMR in SED with a mean bias of 33 kcal/d, with 95% limits of agreement ranging from -337 to 270 kcal/d (*Figure 10*). In athletes, the mean bias for Cunningham was 122 kcal/d, with 95% limits of agreement ranging from -416 to 171 kcal/d. Linear regression between the Cunningham predicted RMR and measured RMR (*Figure 11*) revealed coefficients of determination of R^2=0.58 and R^2=0.59 in SED and athletes, respectively. The slope of the linear regression was m=0.45 (SED) and m=0.50 (athletes), and the intercept was 1,057 kcal/d (SED) and 994 kcal/d (athletes).

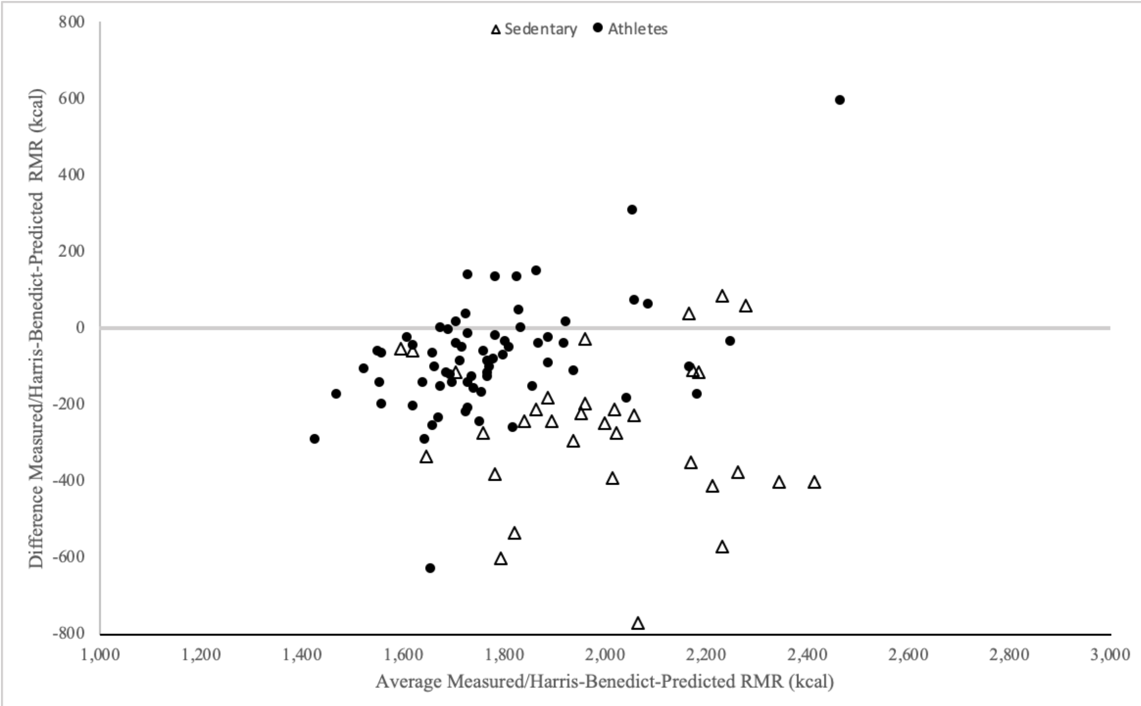

Figure 6. *Bland-Altman plot of measured RMR vs. RMR predicted by Harris-Benedict predictive equation in sedentary controls (open triangles) and athletes (closed circles). The grey line indicates a mean bias of 0 kcal/d.*

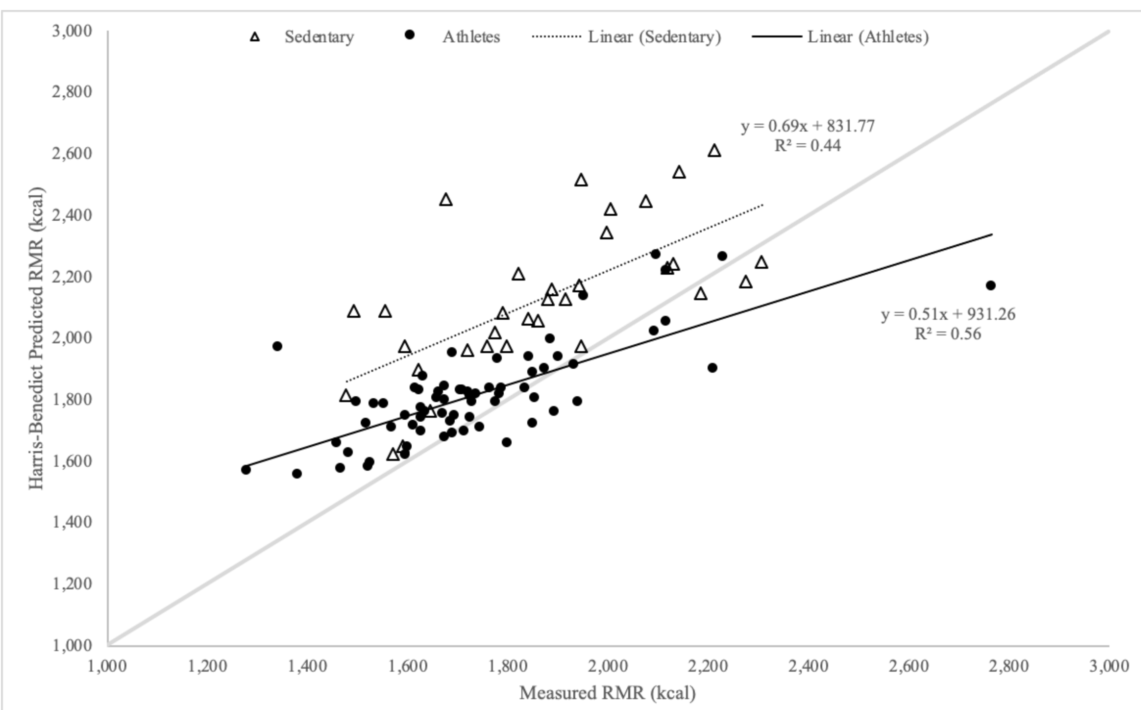

Figure 7. *Comparison of measured RMR and RMR predicted using the Harris-Benedict predictive equation in sedentary controls (open triangles) and athletes (closed circles). The grey line denotes the line of identity.*

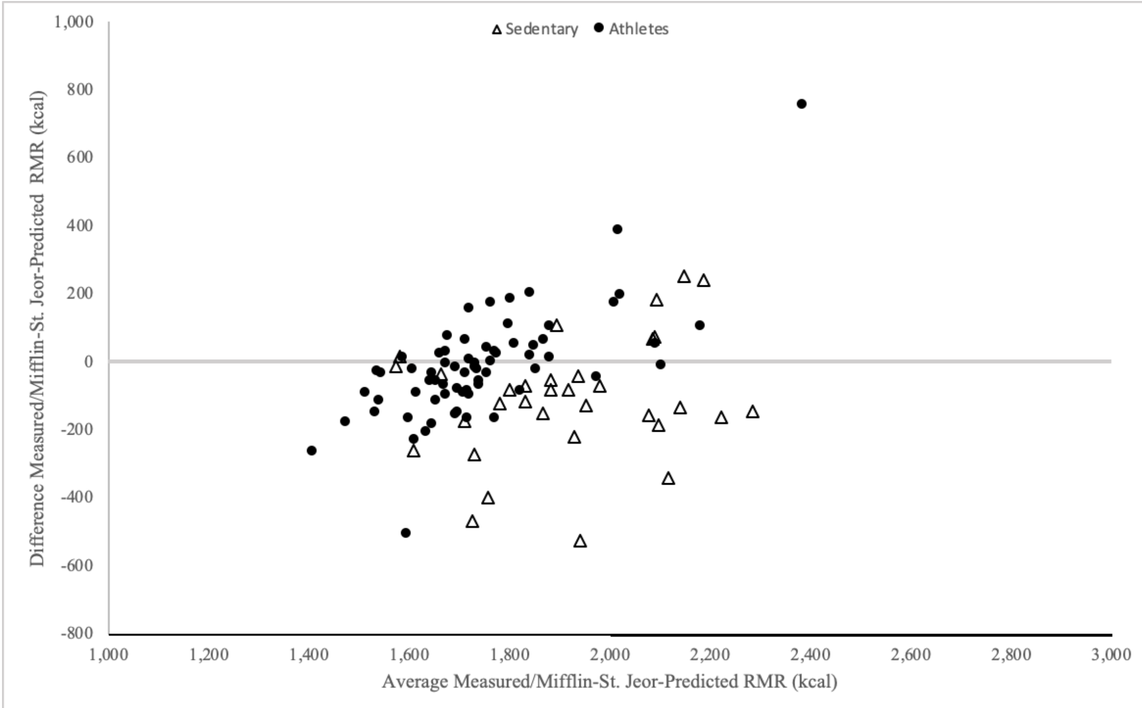

Figure 8. *Bland-Altman plot of measured RMR vs. RMR predicted by the Mifflin-St. Jeor predictive equation in sedentary controls (open triangles) and athletes (closed circles). The grey line indicates a mean bias of 0 kcal/d.*

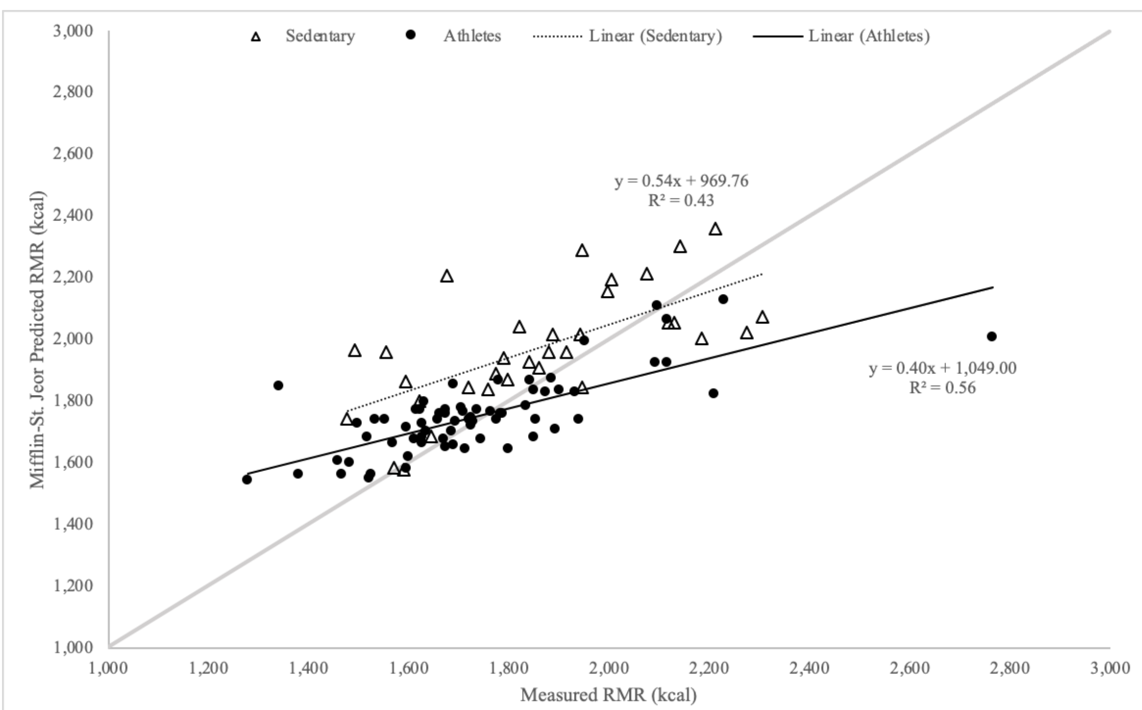

Figure 9. *Comparison of measured RMR and RMR predicted using the Mifflin-St. Jeor predictive equation in sedentary controls (open triangles) and athletes (closed circles). The grey line denotes the line of identity.*

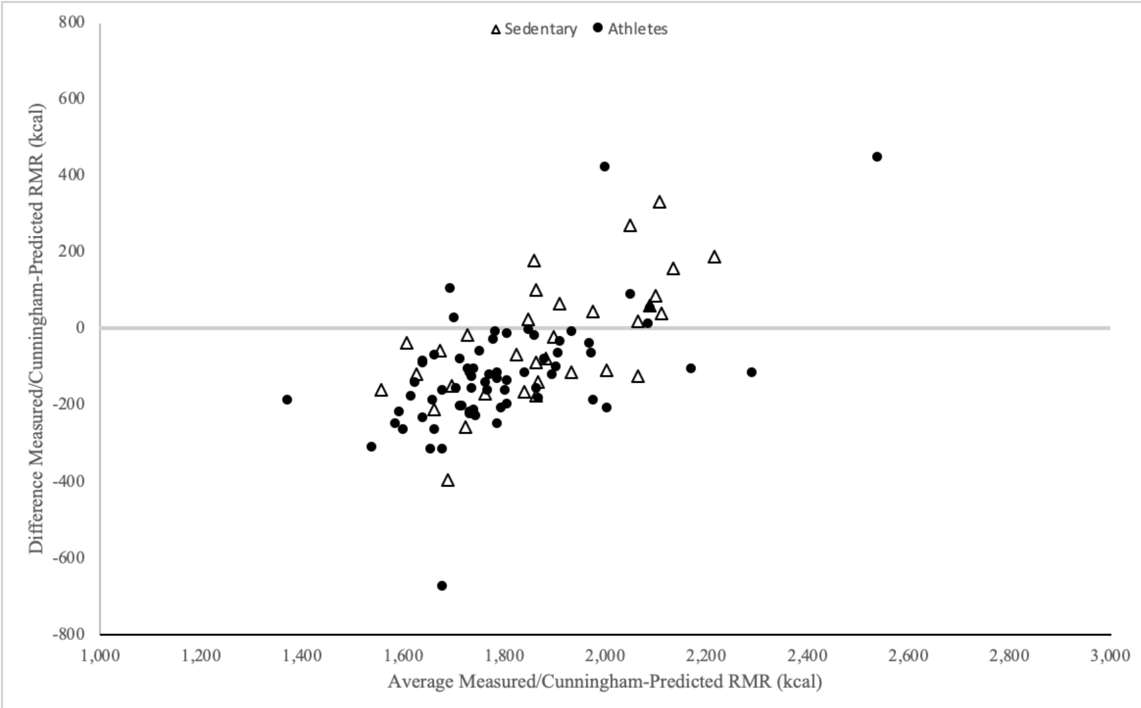

Figure 10. *Bland-Altman plot of measured RMR vs. RMR predicted by the Cunningham predictive equation in sedentary controls (open triangles) and athletes (closed circles). The grey line indicates a mean bias of 0 kcal/d.*

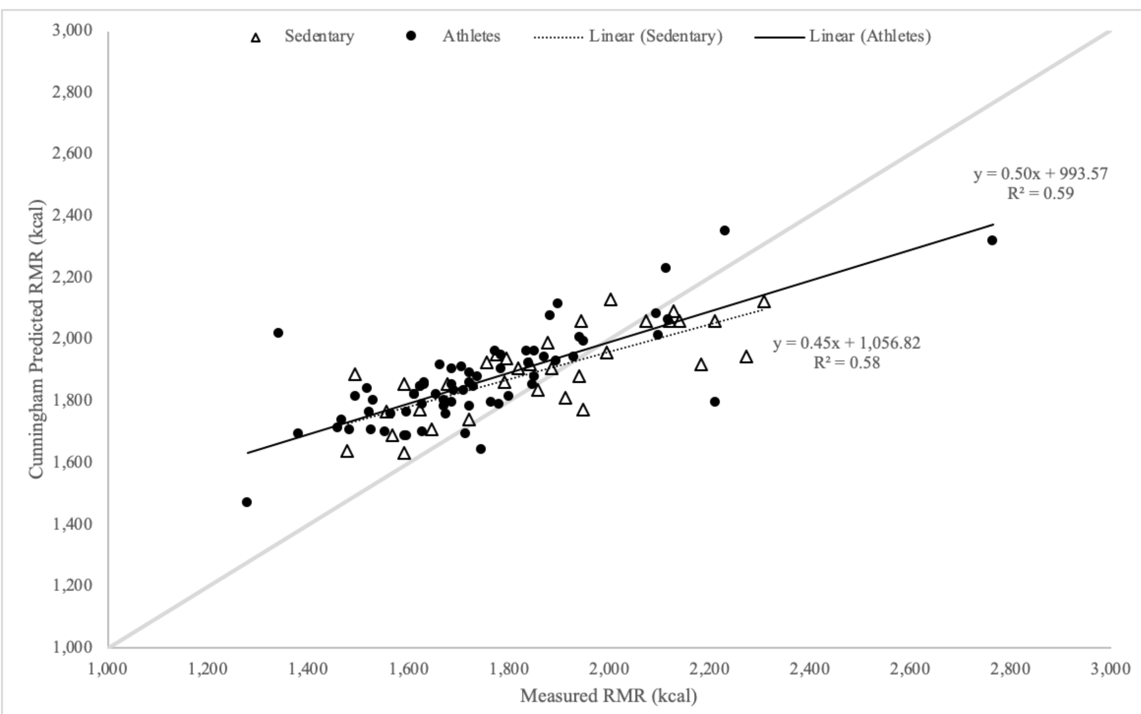

Figure 11. *Comparison of measured RMR and RMR predicted using the Cunningham predictive equation in sedentary controls (open triangles) and athletes (closed circles). The grey line denotes the line of identity.*

Comparison of Measured RMR to Predicted RMR in Sedentary and All Athletes

For SED, the mean bias was lowest in DXA_E, and R^2 was equal (R^2=0.58) between DXA_E and Cunningham predictive equations (*Table 7*). The DXA_E predictive equation predicted RMR in SED within 2 kcal/d, and the Cunningham equation predicted within 33 kcal. DXA_C underpredicted RMR in SED by 92 kcal/d. Both the Harris-Benedict and Mifflin-St. Jeor predictive equations overpredicted RMR in SED by 108 and 262 kcal/d, respectively. R^2 values were similar for SED between Harris-Benedict (R^2=0.44) and Mifflin-St. Jeor (R^2=0.43) predictive equations.

In athletes, the mean bias was lowest in Mifflin-St. Jeor (R^2=0.56), and R^2 was the greatest in DXA_E (R^2=0.60) and Cunningham (R^2=0.59) predictive equations (*Table 7*). The Mifflin-St. Jeor predictive equation predicted RMR in athletes within 14 kcal/d. DXA_E overpredicted RMR in athletes by 37 kcal/d and Cunningham overpredicted RMR by 122 kcal/d. Both DXA_C and Harris-Benedict predictive equations overpredicted RMR in athletes by 124 kcal/d and 78 kcal/d, respectively. R^2 values were equal (R^2=0.56) for athletes between Harris-Benedict and Mifflin-St. Jeor predictive equations.

Table 7. Summary of Equations in Predicting Measured RMR for All Athletes and Sedentary Controls

		Mean Bias	95% Limits of Agreement	R^2	Slope	Intercept
DXA_E	*SED*	**2**	**-301, 297**	**0.58**	0.48	965
	Athletes	37	**-331, 256**	**0.60**	0.49	**921**
DXA_C	*SED*	92	-234, 417	0.51	0.39	1,043
	Athletes	124	-431, 183	0.54	**0.54**	926
Harris-Benedict	*SED*	262	-642, 118	0.44	**0.69**	**832**
	Athletes	78	-379, 224	0.56	0.51	931
Mifflin-St. Jeor	*SED*	108	-457, 242	0.43	0.54	970
	Athletes	**14**	-327, 300	0.56	0.40	1,049
Cunningham	*SED*	33	-337, 270	0.58	0.45	1,057
	Athletes	122	**-416, 171**	0.59	0.50	994

Bolded values are deemed preferential values, indicated as a mean bias value closest to 0 kcal/d; narrowest 95% limits of agreement; R^2 value closest to 1; slope closest to 1; intercept closest to 0

Comparison of Measured RMR to Predicted RMR in Non-Weight-Sensitive and Weight-Sensitive Athletes

In NWS athletes, the best agreement was found in DXA_C, as indicated by an average measured-to-predicted RMR ratio of 1.00 ± 0.14 (*Figure 12*). DXA_E resulted in an average RMR ratio of 1.04 ± 0.14 for NWS athletes. Both Cunningham and Harris-Benedict equations slightly overpredicted RMR in NWS athletes and revealed similar RMR ratios of 0.99 ± 0.14 and 0.98 ± 0.14, respectively (*Figure 13*). Mifflin-St. Jeor slightly underpredicted RMR in NWS athletes and resulted in an average RMR ratio of 1.04 ± 0.15.

In WS athletes, all predictive equations significantly overpredicted measured RMR (Mifflin-St. Jeor, $p=0.009$, all other equations, $p<0.001$). The lowest measured-to-predicted RMR ratio in WS athletes was observed for DXA_C, which was indicated by a ratio of 0.92 ± 0.05 (*Figure 12*). DXA_E resulted in average measured-to-predicted RMR ratio values of 0.96 ± 0.05. Both Cunningham and Harris-Benedict equations overpredicted RMR in WS athletes and resulted in an RMR ratio of 0.92 ± 0.05 and 0.95 ± 0.06, respectively (*Figure 13*). The Mifflin-St. Jeor equation most closely predicted RMR in WS athletes with an average RMR ratio of 0.98 ± 0.06.

The DXA_C predictive equation resulted in the greatest discrimination of measured-to-predicted RMR ratio between NWS and WS athletes (p=0.059). The next predictive equation providing the greatest discrimination between NWS and WS athletes was DXA_E (p=0.094). Cunningham and Mifflin-St. Jeor resulted in similar significance of discrimination between NWS and WS athletes (p=0.115 and p=0.139, respectively), whereas Harris-Benedict provided the lowest discrimination between NWS and WS athletes (p=0.422).

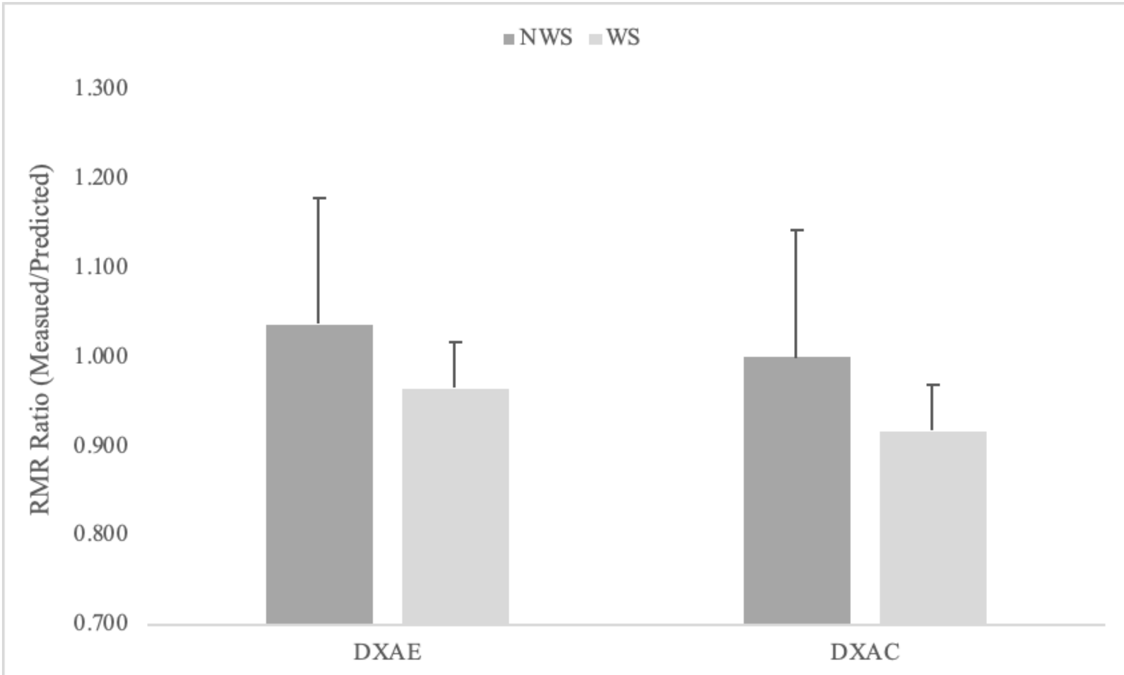

Figure 12. *Comparison of the ratio between measured RMR and RMR predicted using the expanded DXA-predictive equation (DXA_E) and the condensed DXA-predictive equation (DXA_C) in non-weight-sensitive athletes (NWS) and weight-sensitive (WS) athletes.*

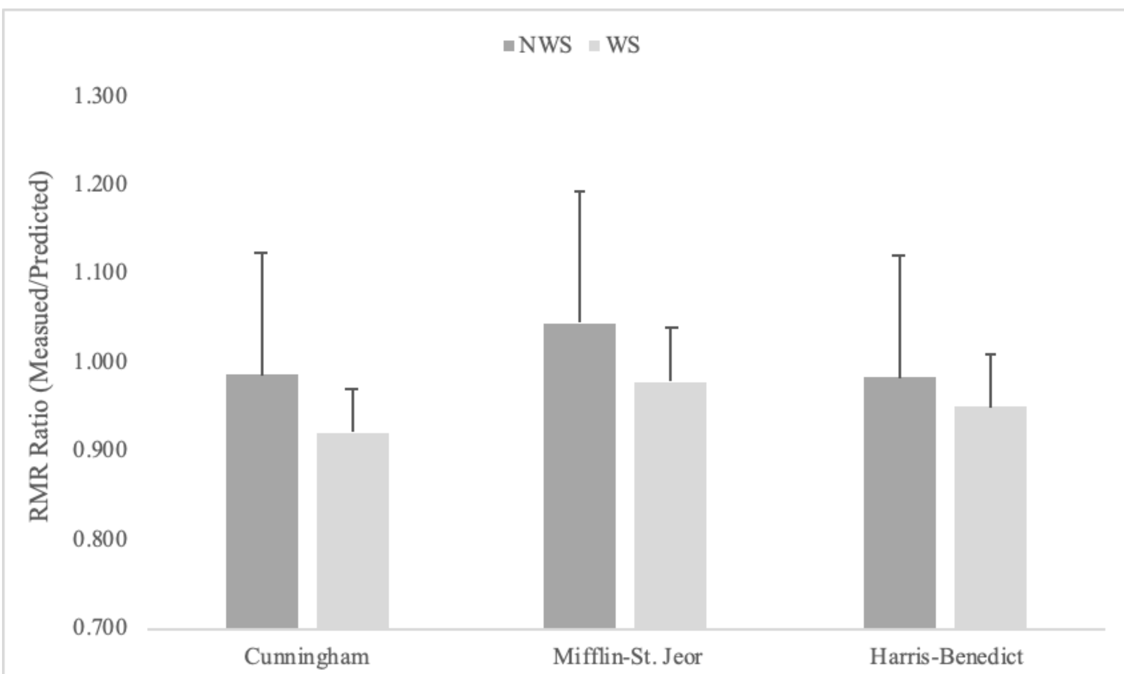

Figure 13. *Comparison of the ratio between measured RMR and RMR predicted using Cunningham, Mifflin-St. Jeor, and Harris-Benedict predictive equations in non-weight-sensitive athletes (NWS) and weight-sensitive (WS) athletes.*

CHAPTER 5: DISCUSSION

The purpose of this study was to test advanced segmental body composition measured by DXA along with established equations and organ-tissue metabolic rate coefficients to predict RMR in athletes. The first aim of the study was to test the DXA-predictive equation method compared to commonly used predictive equations (Harris-Benedict, Mifflin-St. Jeor, Cunningham) to predict RMR in SED and athletes. In relation to this aim, we found the DXA_E predictive equation to more accurately and precisely predict RMR in both SED and athletes compared to other predictive equations. On average, DXA_E predicted RMR within 2 kcal/d for SED and 37 kcal/d for athletes. This predictive equation accounted for the greatest variance compared to all other predictive equations, accounting for 58% of the variance in RMR for SED and 60% of the variance for athletes.

We found the Harris-Benedict predictive equation to consistently overpredict RMR in both participant groups. RMR was significantly overpredicted in SED by 262 kcal/d and 78 kcal/d in athletes. This predictive equation utilizes demographic and anthropometric measurements to predict RMR including age, height, weight, and sex. Although there were not any significant differences in height between SED and athletes, there were significant differences observed between SED and athletes for age and body weight, as SED had a higher mean age and a higher mean body weight compared to athletes. Our findings of overprediction of RMR with the use of the Harris-Benedict equation in sedentary, non-athletic individuals is consistent with the literature (Daly et al., 1985; Mifflin et al., 1990; Owen et al., 1986; Owen et al., 1987). Our study found the Harris-Benedict equation to overpredict RMR by an average of 14% in SED and 4.5% in athletes. Daly et al. (1985) observed the Harris-Benedict equation to overpredict RMR in healthy males and females by an average of 12.3%, with up to 25-30% overprediction in some participants. Another study noted an overprediction of RMR by 5% in normal-weight and obese males and females (Mifflin et al., 1980).

There are differences in body composition between these participant groups that should be taken into consideration when predicting RMR. Even though BMI was higher in SED compared to athletes, LBM was found to be comparable between groups. Additionally, FM and body fat percentage were both significantly higher in SED compared to athletes. The Harris-Benedict equation does not take these body composition measurements into account within the prediction equation, which can partially explain the overestimation of RMR when using Harris-Benedict. Since the DXA_E predictive equation accounts for each tissue compartment within the equation to predict RMR, it is a more appropriate equation to utilize, especially for athletic populations which tend to have a greater proportion of FFM compared to the general population.

Comparison of Validity Between the Expanded and Condensed DXA-Derived Equations

The findings from this study show the DXA_E predictive equation to more consistently predict RMR in both SED and athletes compared to DXA_C. DXA_E provides more desirable measures of validity (*Table 7*), which is likely explained by the categorization of the highly metabolic internal organ compartments within the equation. Both equations consider brain, SMM, bone, adipose, and residual mass. DXA_E separately accounts for the internal organs (kidney, heart, liver, spleen) from residual mass, whereas DXA_C incorporates these internal organs within the calculation of residual mass. The metabolic activity coefficient of residual mass is greater in DXA_C (43 kcal/kg/d) due to the inclusion of these internal organs. Even though the residual mass for DXA_E is lower (6.9 kcal/kg/d), each internal organ is given a respective metabolic activity coefficient that accounts for the high metabolic activity of each organ. The energy expenditure of internal organs contributes approximately 60% of total RMR (Elia, 1992), so by accounting for these internal organs and their respective metabolic rates more specifically, this may contribute to the improved accuracy of RMR prediction with the expanded DXA-predictive equation.

In this study, DXA_E has shown to predict RMR more consistently in SED and athletic populations compared to other predictive equations. Previous studies that have utilized the expanded version of the DXA-predictive equation (Bosy-Westphal et al., 2004; Müller et al., 2015; Wang et al., 2010; Wang et al., 2012) were able to identify specific body composition differences between participant groups and determine associations of metabolic adaptations specific to highly metabolic organ compartments. Other studies that have utilized the condensed version of the DXA-predictive equation (Koehler et al., 2016; Kosmiski et al., 2014; Strock et al. 2019) have assessed athletes or individuals that are experiencing a chronic energy deficit. These studies were able to identify suppression of RMR secondary to a chronic state of energy deficiency but were not able to make assumptions specific to the internal organs.

Segmental Body Composition Measured by Dual Energy X-Ray Absorptiometry

When assessing segmental body composition between SED and athletes, there were no significant differences for bone mass or masses for the internal organs (heart, kidney, liver, and spleen). However, SED was observed to have significantly more total adipose tissue and SMM compared to athletes. This is likely due to the observed differences in body weight between SED and athletes, in which SED had significantly greater total body weight. Whereas athletes had significantly greater brain and residual mass (for both expanded and condensed versions) compared to SED.

In comparing NWS and WS athletes, there were no significant differences for residual mass between groups as predicted in both DXA_E and DXA_C predictive equations. Although not significant, results indicated bone mass and adipose tissue mass were greater in NWS, while brain mass was greater for WS athletes. NWS were found to have significantly greater SMM. The internal organ masses including the heart, kidney, liver, and spleen were all found to be significantly smaller in WS when compared to NWS athletes, which is consistent with findings from Bosy-Westphal et al. (2004) in underweight subjects. Additionally, Müller et al. (2015) observed a reduction in body weight and loss of FFM in healthy, young men to be explained primarily by loss of SMM and organ masses. The loss of organ mass was determined to be more significant for the liver and kidneys, which explained 72% of the mass lost from organs (Müller et al., 2015). These highly metabolically active internal organs have shown to greatly contribute to the prediction of RMR.

Comparison of Other Predictive Equations

The Cunningham equation was found to more accurately predict RMR in SED and athletes compared to Mifflin-St. Jeor and Harris-Benedict equations. The Cunningham predicted RMR within 33 kcal/d for SED. Even though the Cunningham equation was found to overpredict RMR in athletes by 122 kcal/d, this equation resulted in similar precision to that of DXA_E when predicting RMR in athletes. The Cunningham equation accounts for LBM in predicting RMR, whereas Mifflin-St. Jeor and Harris-Benedict predictive equations do not consider body composition. This explains the more accurate prediction with Cunningham compared to the other two anthropometric equations. Additionally, the Cunningham equation accounted for 58% of the variance in RMR for SED and 59% for athletes, which is similar to the variation explained by DXA_E predicted RMR for both SED and athletes.

Use of the Cunningham equation requires an assessment of body composition in order to obtain LBM. This study utilized DXA-derived LBM measurement values for the prediction of RMR for the Cunningham equation; however, body composition measurements can be obtained from methods other than DXA, such as underwater weighing, air-displacement plethysmography, bioelectrical impedance analysis, and skinfold-thickness measurements (Sun et al., 2005). Some of these methods have greater reliability and accuracy compared to other methods (Kirstorp et al., 2000), and this should be taken into consideration when selecting a method for measurement of body composition. Skinfold-thickness assessments are not considered as reliable due to high error probability, and previous literature (Kirstorp et al., 2000; Sun et al., 2005) has shown that bioelectrical impedance analysis is not as accurate in estimating energy expenditure when compared to DXA technology. DXA is considered the gold standard for body composition measurement, but this technology is costly and requires certified personnel in order to operate. If DXA is not available for use to obtain measurements of LBM, another method can be utilized, but it should be considered that the Cunningham equation may potentially yield less accurate results depending on the method utilized to obtain LBM. Additionally, the population should be considered when selecting a body composition measurement method in order to reduce potential error as much as possible.

Detection of Energy Deficiency in Athletes

The second aim of this study was to test if the DXA-predictive method could be used for the detection of RMR suppression in athletes experiencing LEA. In the current study, DXA_C was found to result in the greatest discrimination of measured-to-predicted RMR ratio between NWS and WS athletes. This DXA-predictive equation significantly overpredicted RMR in WS athletes, who are assumed to be in an energy deficient state. The DXA_C predictive equation provided the lowest measured-to-predicted RMR ratio in WS athletes, indicated by a ratio of 0.92. Additional studies have utilized DXA technology to calculate measured-to-predicted RMR ratio in order to assess energy status in athletes (Koehler et al., 2016; Strock et al., 2019). Strock et al. (2019) determined that predictive equations which utilized DXA-derived measurements are better at identifying athletes with reduced RMR compared to other anthropometric-derived predictive equations. Their findings suggested a threshold ratio value of 0.90 or less as an indicator of metabolic suppression secondary to energy deficiency, although this ratio could be as high as 0.94 when utilizing DXA measurements (Strock et al., 2019). This data suggests that the WS athletes for this study could potentially be experiencing a state of energy deficiency; however, this RMR ratio method needs to be further assessed in energy deficient athletes.

Study Limitations

One limitation of the present study is the significant difference in body composition and weight between SED and athletes. Both BMI and weight were significantly higher in SED compared to athlete participants; however, LBM was similar between groups by design, as participants for the SED group were selected as controls for the study based on similarity of LBM proportion comparable to athlete participants. Unlike Harris-Benedict and Mifflin-St. Jeor, Cunningham accounts for LBM when predicting RMR. LBM, as well as other components of FFM, are also considered in both DXA-derived equations. Therefore, the proportion of LBM for SED participants was of greater importance for the aims in this study compared to overall body weight.

The mean age was also significantly higher in SED compared to athletes, suggesting a potential decline in RMR for SED. However, SED reported a higher measured RMR compared to athletes. As other studies have reported (Cunningham, 1980; Elia, 1992; Owen et al., 1987) age does not correlate with RMR as strongly compared to body weight and body composition. An observed decline in RMR due to age is presumably explained by the changes in body composition or more specifically FFM, which greatly influences RMR, rather than solely dependent on the variable of age alone. Therefore, the decline in FFM rather than difference in age may at least partially explain the overprediction of RMR in SED with both the Harris-Benedict and Mifflin-St. Jeor equations. Both the Harris-Benedict and Mifflin-St. Jeor equations account for age in predicting RMR; however, the equation coefficients for age are rather minimal and likely do not impact mean RMR prediction as greatly compared to body weight.

Only male participants have been included within the present study. Inclusion of female participants may have potentially resulted in different findings, specifically related to body composition between participant groups and how this potentially impacts predicted RMR. Females tend to have a greater composition of FM compared to males, especially in the extremities (Müller et al., 2001), whereas males tend to have greater FFM (Mifflin et al., 1990). Müller et al. (2001) found that LBM accounts for 74% of trunk weight in females, with an even greater contribution at 83% in males. Previous studies have found that females tend to have lower measured RMR when compared to males (Müller et al., 2001; Thompson & Manore, 1996), which is likely due to the difference in body composition between sexes. Lower measured RMR values would have likely been observed in female participants, which can be attributed to less overall FFM compared to males. Additionally, differences in RMR predicted via DXA-derived equations would have resulted due to the differing proportions of LBM within the trunk.

Participant group sizes are not equal between groups (SED n=33, all athletes n=68, NWS=13, WS=55), with only approximately one-third of the participants enrolled as SED controls. There is an even larger discrepancy between the number of NWS and WS athletes enrolled in the study. There was difficulty recruiting an adequate number of NWS athletes for the study, and in order to have enough participants, other recruiting locations were involved in data collection for the study. Data collection occurred at various sites, and more specifically in different counties. There were a small number of participants recruited in the United States; however, a larger sample was needed for the study. This was the option which best allowed for a larger sample size. In order to address this limitation, study protocol was standardized across all sites and all trained personnel utilized equipment according to manufacturers' guidelines.

Although indirect calorimetry is commonly used to determine RMR, this method is not without error. The variation caused by this method has been found to be greater than 4%, with precision of 4.4 to 6.5% (Mueller et al., 2001). Compher et al. (2006) reports interindividual variance of 3 to 5% over a 24-hour period and up to 10% variation over a period of weeks or months. Many studies utilize this data collection method; however, researchers do not necessarily follow the same protocol standards. Study protocol is often standardized in order to reduce the introduction of error from thermic effect of food or activity prior to the assessment. However, there have been discrepancies in the measurement time of gaseous exchange rate, as well as the time in which steady state data is collected for analysis. It has been suggested that a minimal rest period of 10 to 20 minutes is ideal before initiating RMR measurement in healthy adults (Compher et al., 2006). Additionally, in order to obtain accurate measurements, attention should be given to ensure steady-state conditions. It is recommended to discard the initial 5 minutes, then measure continuous gas exchange for at least a 5-minute period in which there is 10% or less variation of oxygen and carbon dioxide exchange (Compher et al., 2006).

DXA technology has been utilized more frequently over the past several decades; however, there are still potential errors associated with this method. It has been found that the interindividual error for body fat is 1.2% in DXA measurements (Wang et al., 2010), with reported error measurements of 3% for body fat, 1.2% for BMC, and less than 1% for FFM in repeated measurements (Tinsley et al., 2018). One source for error specific to this study is that the body composition measurements occurred at various sites. In efforts to minimize this error, all DXA scans were performed on equipment from the same manufacturer by certified personnel. All DXA scans were reanalyzed for each participant by the same individual using the same software. Additionally, this study utilized previously published equations (Bosy-Westphal et al., 2004) in order to estimate organ mass. Organ mass is ideally measured with MRI technology for more accurate and precise predictions of RMR rather than the use of predictive equations to estimate organ mass. MRI-derived measurements would have provided more precise organ mass for participants; however, this technology was not readily available for this study.

Another study limitation is the assumption of energy deficiency in WS athletes. As previously described, athletes may display signs of LEA but still present with an appropriate body weight (Logue et al., 2018; Melin et al., 2019). There is not currently a gold standard for diagnosing chronic energy deficiency in athletes, as there are many potential metabolic adaptations that can occur in response to LEA (Heikura et al., 2018; Koehler et al., 2016; Mountjoy et al., 2014; Strock et al., 2019). In addition to suppression of RMR, disturbances in menstrual cycles and hormone levels have been researched as a metabolic adaptation. Several studies (Heikura et al., 2018; Koehler et al., 2016; Logue et al., 2018; Melin et al., 2019; Strock et al., 2019; Trexler et al., 2014) have researched these adaptations in response to energy deficiency in athletes. Our study did not measure hormone levels of participants and was therefore not able to make any assumptions of LEA in regard to hormone reduction for the athletes participating in this study. However, measured-to-predicted RMR ratio has been more recently utilized as a potential indicator of energy deficiency in several studies (Koehler et al., 2016; Strock et al., 2019). This method should be further assessed in future studies in order to determine the most accurate threshold ratio for energy deficiency.

Conclusion

Body composition has been repeatedly shown to be the best indicator in predicting RMR. Therefore, more detailed body composition measurements available for an individual will result in more accurate predictions of RMR. It is important to utilize DXA technology if possible, as this method has been shown to obtain the most precise measurements of body composition (Kirstorp et al., 2000; Sun et al., 2005). However, there are situations in which DXA is not available for use. In these instances, the Cunningham predictive equation would be recommended. The variance is the same for SED between DXA_E and Cunningham, and there is only an additional 2% variance explained via DXA_E for athletes. LBM measurement is required for Cunningham and can be obtained through other body composition measurement methods. However, depending on the accuracy of the selected method for measuring LBM, this can potentially result in a less accurate RMR measurement predicted by Cunningham.

The data from this study presents beneficial results for both practitioners and researchers. Practitioners should consider the population when utilizing predictive equations to estimate energy requirements for a patient. The expanded DXA-derived predictive equation has shown to be the most precise predictive equation for sedentary individuals or non-athletes. As an alternative, the Cunningham predictive equation should be utilized, as both the Harris-Benedict and Mifflin-St. Jeor equations tend to overestimate RMR in sedentary and obese or overweight individuals. Specific to athletes, the expanded DXA-derived predictive equation has shown to most accurately predict RMR. If DXA technology is not available for use, the Cunningham predictive equation will be most appropriate, as it has shown to predict RMR more precisely in athletes compared to other predictive equations that utilize anthropometric-derived measurements.

It has been established that LEA results in many detrimental effects on an athletes' health status and sport performance (De Souza et al., 2019; Logue et al., 2018, Melin et al., 2019, Mountjoy et al., 2014). It is difficult to assess energy availability, as there is not currently a standardized method to assess energy deficiency in athletic populations (Logue et al., 2018; Strock et al., 2019). All predictive equations assessed in this study were found to significantly overpredict RMR in WS athletes. Calculation of measured-to-predicted RMR ratio is one potential indicator of energy deficiency that can be utilized in athletes. These study results indicate the need for further validation of the expanded DXA-predictive equation in athletic populations. The variance in predicted RMR should be further examined to explain the individual differences. There are many possible explanations resulting in individual variations of RMR such as hormone levels and dietary intake, neither of which were measured for this particular study.

In summary, the results of this study demonstrate that DXA_E provides the most accurate predictive equation for SED, whereas the DXA_E and Cunningham equations have both demonstrated to be reliable equations for athletic populations. These equations should both be used with caution for athletes participating in weight-sensitive sports, as this has not adequately been shown to be the best predictive equation for these athletes. According to our findings, Harris-Benedict and Mifflin-St. Jeor equations are not appropriate for athletic populations, especially those assumed to be in an energy deficit, as they tend to significantly overpredict energy requirements in these populations.

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